

Lund 1980

**EFFECTS OF IRRADIATION
ON THE SMALL INTESTINE OF THE RAT
A SEM STUDY**

LARS-GÖSTA FRIBERG



EFFECTS OF IRRADIATION
ON THE SMALL INTESTINE OF THE RAT
A SEM STUDY

From the Department of Oncology
University of Lund
Sweden

EFFECTS OF IRRADIATION ON THE SMALL INTESTINE OF THE RAT A SEM STUDY

LARS-GÖSTA FRIBERG



Lund 1980

THESIS AT LUND UNIVERSITY
ISBN 91-7222-298-0



The investigation has been supported by grants from Agni Olssons
and Sigrid Simonssons fond and from John and Augusta Perssons fond

Printed in Sweden
Berlings, Arlöv 1980

Contents

CHAPTER I	INTRODUCTION	7
CHAPTER II	GENERAL REVIEW	9
CHAPTER III	MATERIAL AND METHOD	11
	A. Experimental model	11
	B. Irradiation technique	12
	C. SEM-preparation	13
CHAPTER IV	SEM OF THE NORMAL SMALL INTESTINE	15
	A. Fasting animal	17
	B. Non fasting animal	31
	C. Bacteria	38
CHAPTER V	ARTIFACTS	43
CHAPTER VI	THE SCORE SYSTEM	47
CHAPTER VII	SINGLE DOSE IRRADIATION	55
	A. Introduction	55
	B. Initial reaction	57
	20 Gy 59 – 25 Gy 63 – 30 Gy 69	
	C. Early reaction	71
	2 Gy 73 – 3 Gy 76 – 6 Gy 77 – 9 Gy 80 – 12 Gy 84 –	
	15 Gy 86 – 20 Gy 90 – 22.5 Gy 95 – 25 Gy 101 – 30 Gy 109	
	D. Repair	115
	15 Gy 117 – 20 Gy 119 – 22.5 Gy 124	
	E. Score curves	127
	F. Body weight	133
	G. Discussion	139
CHAPTER VIII	FRACTIONATED IRRADIATION	145
	A. Introduction	145
	B. 5 F/week	147
	C. 2 F/week	171
	D. 15 F/week	181
	E. 3 F/week	193
	F. 1 F/week	203
	G. Weight curves	215
	H. Discussion	221
CHAPTER IX	SUMMARY	227
	CODE OF MAGNIFICATIONS	229
	REFERENCES	231

Chapter I

Introduction

Since megavoltage irradiation was introduced in the 1950's, the better depth dose and the more homogeneous distribution of the dose have resulted in increased 5-year survival for patients with pelvic tumors. In advanced stages of carcinoma of the cervix, the treatment with intracavitary radium does not give an adequate tumor dose in the peripheral sections close to the pelvic wall due to the

low intensity from the irradiation source, depending on the inverse square law. Complementary X-irradiation could neither give a dose high enough to the pelvis without causing side effects to the skin. On the other hand, the cobalt-60 technique also has disadvantages. As seen in Fig. 1, showing a conventional irradiation field of $15 \times 15 \text{ cm}^2$, the major part of the small intestine is situated within the irradiated



Fig. 1. A major part of the small intestine is situated within a conventional field in pelvic irradiation.

volume and thus gets the same dose as the tumor. Further on, in the opposing field given irradiation with cobalt-60, when the lymph nodes are treated and given 100%, the isodose for 120% may include part of the small intestine. Using the linear accelerator or the betatron, this problem does not appear, but still the tumor and the small intestine get the same dose, which cannot be avoided.

The small intestine is a highly radiosensitive tissue with high mitotic activity in the lower part of the crypts of Lieberkühn and a short turn-over time, 36–48 hours. As in other tissues three basic types of cells are involved: the stem cells of the crypt, the functional cells and the supporting stroma cells. The proliferation capacity in the crypts is enormous: with one single cell surviving irradiation, repopulation and repair of a villus are possible (Quastler, 1959).

Clinically, the primary radiation reaction starts during or in connection with the treatment, giving symptoms as nausea, vomiting and diarrhoea. The secondary or late reaction, e.g. fibrosis and obstruction of the intestine, may give clinical symptoms after one to two years or more. The relationship between early and late reaction is not clear. An early damage to the vascular bed may be the explanation. A lower degree of complications has been reported after mechanical or pharmacological reduction of the blood supply to the intestine (Larsson and Sténson, 1965; Penn *et al.*, 1975). This hypoxia is one way to solve the problem. Low dose rate irradiation may be another method to minimize the side effects to the gastrointestinal tract (Cassady *et al.*, 1975).

A lot of clinical reports describe various complica-

tions of the small bowel where a progressive obliterative endarteritis and a submucosal fibrosis may be the morphological injury. (Deveny *et al.*, 1976; Maruyama *et al.* 1974; Green *et al.* 1975; Joelsson *et al.*, 1971; Newman *et al.*, 1973; Roswitt *et al.*, 1972; Mason *et al.*, 1970; Dencker *et al.*, 1971). A broad review of the complications was also given by Rubin and Casarett, (1968). Irradiation may also result in disturbances of the intestinal function. Morphological damage gives malabsorption syndromes such as reduced glucose absorption and a reduced absorption of oleic acid, iron, vitamin B₁₂ (Greenberger, 1964; Reeves *et al.*, 1965; Goldberg, 1972). Leakage of protein has been observed by Vatistas and Hornsey (1966). A damage to the brush border may result in a reduction of disaccharidase-activity (Tarpila, 1971; Becciolini *et al.*, 1974). Another effect of irradiation described, is disturbances of the motility, (Swift *et al.*, 1955; Frankendahl, 1973).

In radiotherapy, the total dose that can be given to the pelvic region lies between 45 and 55 Gy, depending on the size of the irradiation field and the fraction size (Strockbine *et al.*, 1970). The risk for small intestine ulceration, perforation, fibrosis or obstruction is increasing to a risk level of 25–50% when the total dose approaches 60 Gy (Roswitt *et al.*, 1972).

The aim of the following investigation has been to simulate the conditions in radiotherapy, examining the effect of partial abdominal irradiation in rat, by SEM-technique. Different fractionation schedules were tested and compared with the effect of single dose irradiation. As control LM and TEM were used.

Chapter II

General review

The literature concerning the experimental studies of the irradiation effects on the small intestine is extensive. The highly proliferative tissue with a short life span of the cells and the importance of the function of the mucosa have resulted in vast studies covering radiobiology, radiotherapy, bacteriology and gastroenterology.

Beside clinical reports on reaction following X-ray treatment, local intracavitary-treatment and external high voltage treatment the experimental studies of animals are dealing with the effect on the crypts of Lieberkühn in cell kinetics and the functional disturbances of the intestinal villi.

The change in the number of cells in the crypts or in the migration to the villus may be followed by counting the cells in histological preparations (Warren and Whipple, 1923; Friedman, 1945; Devik 1971), or by using different labelling techniques and follow the DNA-synthetizing cells (Lesher, 1967; Hagemann *et al.*, 1971; Wiernik, 1971; Lesher *et al.* 1975). Light microscopy as well as transmission electron microscopy have been used (Hampton, 1966; Tarpila, 1971). Usually whole-body and single dose irradiation studies have been used, but also abdominal irradiation, as well as irradiation on exteriorized intestine and fractionated irradiation have been analyzed (Lesher *et al.*, 1975; Withers, 1975). The effects of fractionated irradiation in human beings have also been studied by means of biopsies from the small intestine of patients (Wiernik, 1966). Most of the results, however, concern dose-time relationship of the damage of the crypts and the mechanisms of changes in the cell proliferation.

The effect on the mucosa, especially the villi, is well known from the classic work of Cronkite, (1960), who shows that the villus retracts and that a single dose of 30 Gy results in a shortening of the villus within a few days to a total disappearance. The result is a flat surface covered not by the normally columnar epithelial cells, but by thin so-called omega cells of squamous cell type. In the next stage, this cell layer has been lost and the end result

is an ulcerated surface. During the irradiation there is a kind of feed-back mechanism between the damaged crypt and the villus, where an extrusion of cells is going on, determined by an equilibrium between crypt and villus (van der meer fieggen, 1973; Rijke, 1974). The causes of death as the final result of the reaction after irradiation are well defined. (Quastler, 1956).

Our knowledge of bacteriology and digestive and absorptive functions in relation to irradiation has increased extensively during the last years. A broad review was presented by Sullivan, (1966). Cell reactions in relation to cell population kinetics and enzyme activities after irradiation have also been studied (Tsubouchi and Matsuzawa, 1973). The surface of the mucosa has not attained the same interest as the cell kinetics or the functional disturbances. Using routine histological technique, it is difficult to fix the crypt and to visualize the villus in desired orientation. With a three-dimensional study, the difficulties can be overcome. (Fujii, 1972).

On the other hand, some techniques, the macro- and micro-colony counting methods, (Withers and Elkind, 1970), make it possible to count the developing crypts and the resulting crypts after irradiation in a transverse section of the intestine. However, the sensitivity of these methods is not appropriate for studies of the effects of irradiation used in the clinic, (Hamlet *et al.*, 1976).

Scanning electron microscopy (SEM) is claimed to be a sensitive method to study surface structures. (Carr and Toner, 1968; Demling *et al.*, 1969; Marsh and Swift, 1969; Toner and Carr, 1968; Balcerzak *et al.*, 1969; Toner *et al.*, 1970; Anderson and Taylor, 1969; Carr *et al.*, 1974). Changes, as a result of the effects of ionizing irradiation, especially of the small intestine which, due to the rapid proliferative activity of the crypt cells and radiosensitivity of that compartment, have all qualifications to be a good indicator of the response to irradiation.

Carr and Toner, (1972) described the effect of 10 Gy single dose given as whole body irradiation to

mice, where SEM revealed the shortening of the villi down to small irregular excrescences 90–100 hours after irradiation. They also found infestation of the intestine by flagellate parasites in this late stage of reaction. The SEM-pictures had an enlargement of $80\times$ to $1600\times$.

Anderson and Withers, (1973), investigated the ileal mucosa by SEM after 10 Gy single dose cobalt-60 whole body irradiation of rats. The greatest degree of effect was observed 3–4 days after irradiation. They found the villi completely reconstructed 5–6 days later. No appreciable effect was notable during the first two days after irradiation. 4 days later they saw densely packed epithelial cells when the crypts were very active. The advantages of using SEM in combination with light microscopy was stressed. SEM was able to give information from a large surface. They used an enlargement below $1000\times$.

Hamlet et al., (1976) compared the effects of cobalt-60 and D-T neutron irradiation, on the mouse intestinal mucosa given as single dose whole-body irradiation. SEM-reaction was related to the crypt-counting technique according to *Withers and Elkind*, (1970). Different single doses were given and dose-response curves for crypt-cell survival and the appearance of the intestinal surface compared. SEM

was judged as the most sensitive method and a difference could be seen between the effects of cobalt and neutrons. The villi were more conical when irradiated with neutrons than with 60-cobalt. 3 Gy gave slight distortion of the villi. This dose is only a third of that necessary for significant change, as observed by the crypt-counting technique. This shows that SEM is a more sensitive method than crypt counting. In conclusion, the authors claim that “mucosal morphology is the total expression of many different biological parameters; the regenerative ability of the crypt epithelium is only one of them”. The enlargement used in SEM was $100\times$. Another study by *Carr et al.* (1979), showed a lack of correlation between the surface of the villus and crypt damage in irradiated mouse intestine.

Porvaznik et al. (1979) studied, using SEM, the density of the indigenous filamentous microflora on the epithelial surface of the rat after whole-body irradiation with 5 Gy and 10 Gy 60-cobalt. After 5 Gy, the bacteria disappeared, but returned again after 2 days. After 10 Gy, they also disappeared but did not return to the mucosal cells. Conclusion: no bacteria were seen after a high dose and thus absorbed products of intestinal bacteria (endotoxin) are responsible for the lethal outcome, and not an increased attack by bacteria directly.

Chapter III

Material and method

A. Experimental model

Sprague and Dawley rats, females, weighing about 200 g have been used throughout the investigation. They were kept two per cage and had free access to water and commercial pellets. Each animal's weight was controlled daily. A plaster model of the animal was used as a model for the construction of two identical perspex casts, i.e. one ventral and one dorsal part, which when put together formed a tightly fitting whole-body cast (Fig. 2(a)). This cast was placed on a stand constructed to permit irradiation from the dorsal and the ventral sides of the rat by turning it 180°. The stand also permitted backscatter-

ing medium to be inserted under the animal for assuring a good definition of dose distribution (Fig. 2(b)). Openings (4×3 cm) were cut in each half on the perspex cast, corresponding to the treatment field, allowing irradiation from opposite sides. A stretched net closed the opening. The proportion between the irradiated volume and the body weight was approximately the same in the rat and in the human being given treatment for cancer in the pelvic region. The larger field in the rat was chosen to assure that the small intestine was situated within the field in the way seen in the X-ray picture from the clinic (Fig. 1). In order to confirm that the position of the intestine was within the beam, measurements in form of

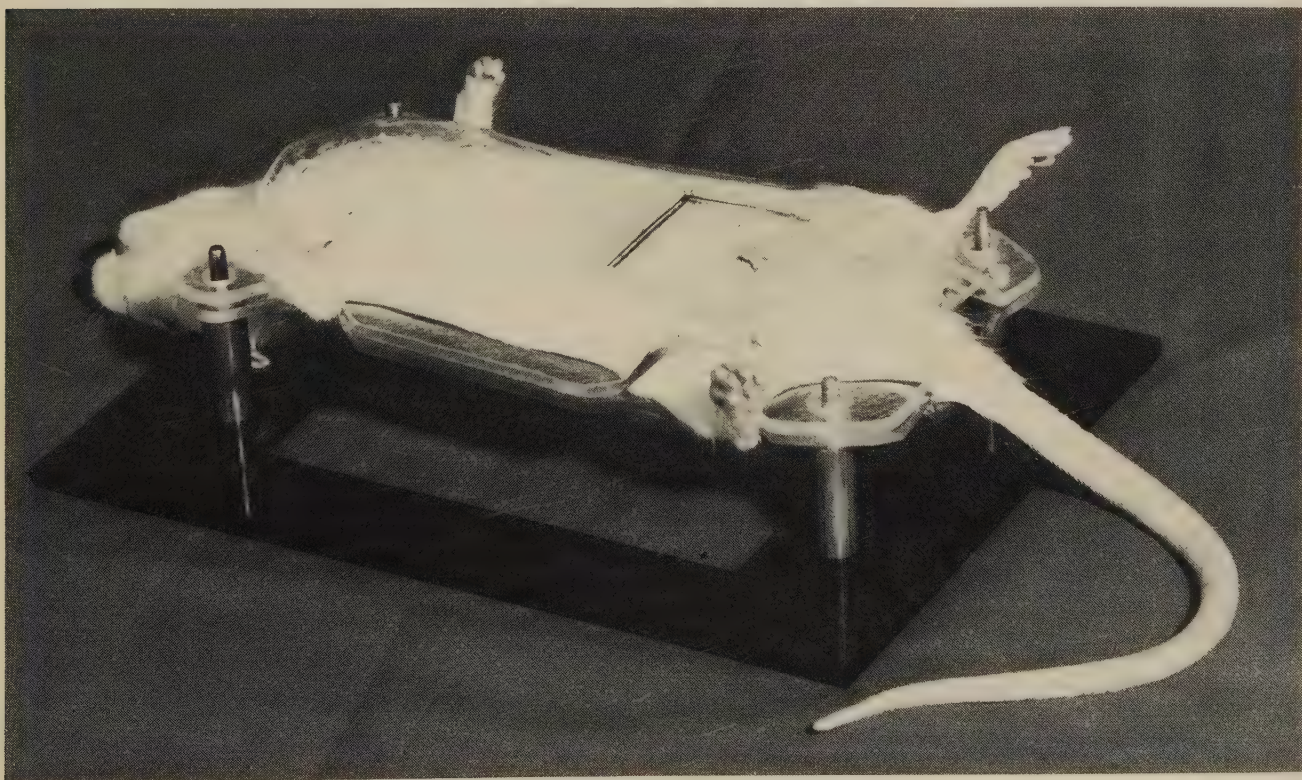


Fig. 2 (a). Perspex chamber with the rat in irradiation position. The opening in the cast is the irradiation field.

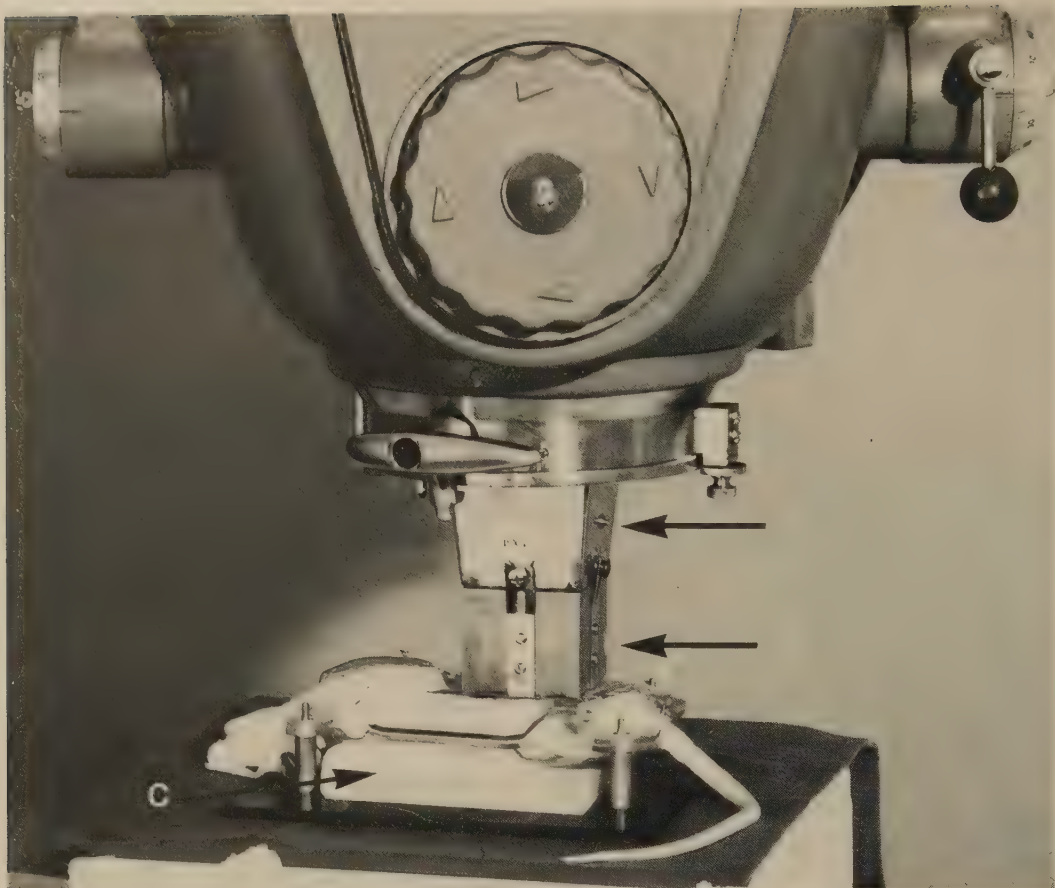


Fig. 2 (b). Irradiation source with collimators and back-scattering material beneath the animal. a. standard collimator. b. added collimator. c. paraffin block.

repeated radiography with barium contrast were performed (Fig. 3). To ascertain that different positions of the rat did not displace the small intestine, silver clips were fixed by operation to the part of the ileum where specimens were taken. X-ray control confirmed that only minor changes in the position of the clips could be reached by shaking the rat with the head upside down or in other positions. Before irradiation the anesthetized rat was always shaken with its head up before bringing the animal into position in the experimental chamber. Nembutal[®], 60 mg/ml, 0.10 ml intraperitoneally, was used for anesthesia, given about 10 minutes before irradiation. The effect lasted for 1–2 hours.

B. Irradiation technique

A cesium-137 therapy unit (Picker[®]) was used as radiation source. Fig. 2(b). A collimator was made for the irradiation field on the constructed model giving a field size of 3×4 cm, to be adapted to the standard collimator. SSD was 20 cm. The choice of cesium-

137 together with a short SSD has the advantage of being a high energy quality (0.66 MeV) but with relatively low depth dose. The close contact of the collimator with the animal gives a penumbra comparable to that in clinical work (8 MV X-ray). The AP-distance of a rat is 3.2 cm and about 20 cm in a patient, which gives the following depth dose from one field in the center of the rat and of the patient: 79% and 73%, respectively. This last value corresponds to a field size of 15 by 15 cm² at a SSD of 100 cm and at 8 MV X-ray. The calculated depth dose in the rat was checked with ionizing chambers by phantom measurements within the plaster model. Four measured values gave $78 \pm 3\%$ and a control with TLD within the abdominal cavity of the rat confirmed the homogeneously absorbed dose within the irradiated volume. The dose rate in the center of the rat was about 0.5 Gy/min. In a patient the dose rate is about 1.4 Gy/min with 8 MV X-ray and 0.7 Gy/min with a cobalt-60 unit. At the beginning of the experiments 0.89 minutes of irradiation from the ventral and the dorsal side gave an absorbed dose of 1 Gy in the center of the rat.

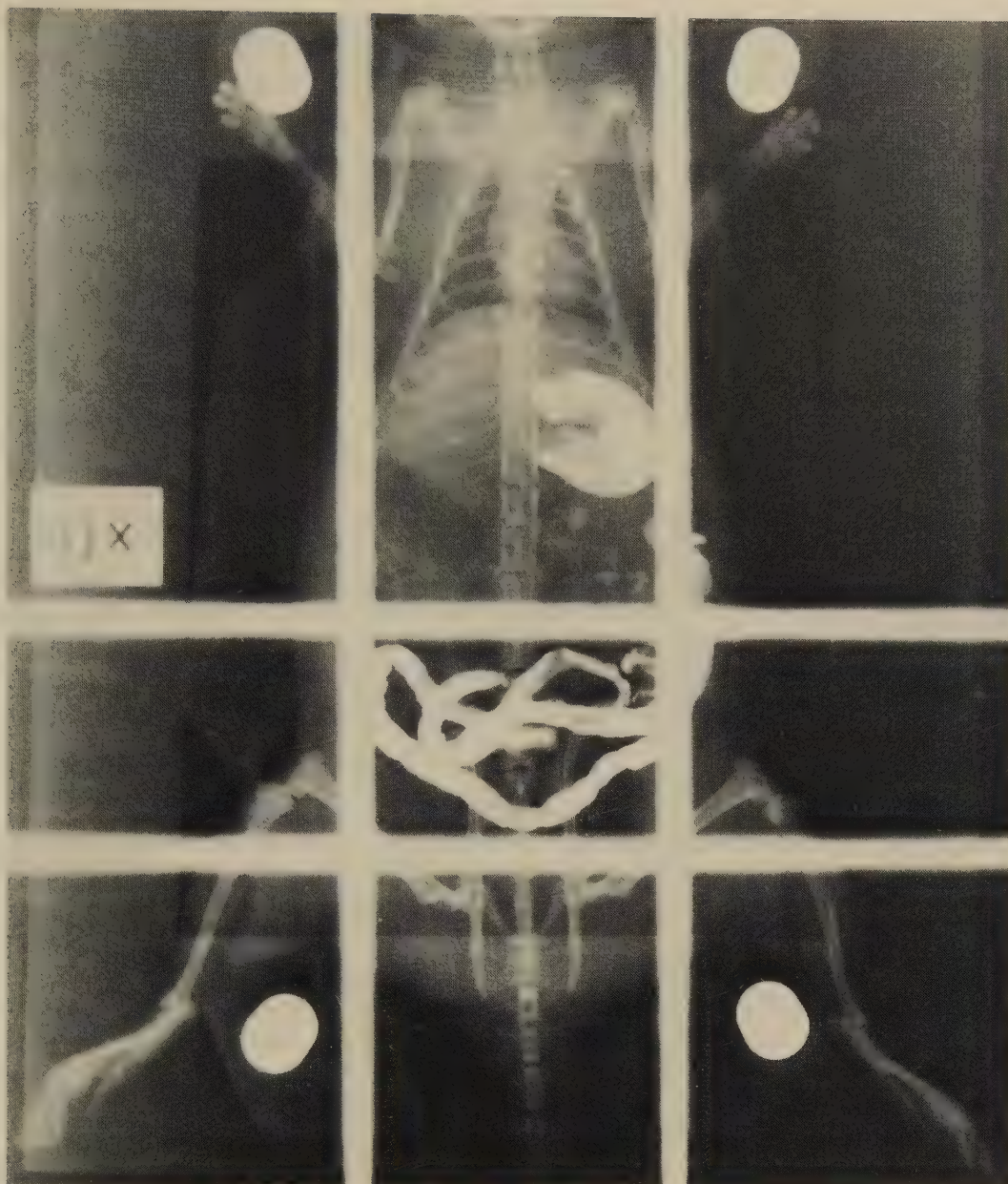


Fig. 3. Irradiation field with barium contrast in the small intestine.

C. SEM-preparation

The abdomen of the rat was opened in the midline under ether narcosis. Ten specimens, 0,5–1,5 cm segments, were taken from the lower ileum and from the upper jejunum outside the treatment field for scanning-electron microscopy and for light microscopy as control. In a few cases samples for transmission electron microscopy were taken to confirm the findings by the other techniques. As mentioned, the weight was controlled daily.

The pieces were cut along the mesenteric border. As a rule they immediately turned inside-out spontaneously, with the mucosa as the convex surface.

To clean the intestine, all pieces were rinsed in three different baths of cold isotone NaCl. Different fixation methods were tested until 2% glutaraldehyde buffered to pH 7.3 at 4°C for 2 hours was considered acceptable (Hopwood, 1967; Arborgh *et al.* 1976). After rinsing, the pieces were kept in cacodylate buffer for up to 24 hours, but mostly all preparations were made directly after fixation. The mucus layer on the surface did not disappear after simple washing in this study, which is also reported by Toner and Carr, (1968), Demling *et al.*, (1969). This mucus layer makes it impossible to see the single epithelial cells or the microvilli. Different enzymes do not remove the mucus layer, (Pfeiffer,

1971). *Balcerzak et al.*, (1969) and *Doran and Heydon* (1971) reported that clean specimens could be obtained by shaking them in 1% HCl, and that this did not result in any morphological changes. The method was tested in this study with varying concentrations of HCl. Thus, all samples were, after fixation, shaken in 1% HCl for 30 seconds, after which they were repeatedly rinsed in NaCl. Dehydration started according to the following scheme: Acetone solutions of 20%, 40%, 70% and 100%, thereafter acetone-amylacetate 1:1 and finally amylacetate alone, followed by air-drying in a chamber. Each step in the dehydration process lasted 30 minutes. Air-drying had to be slow, at least 24 hours. The specimens were controlled from time to time in a dissection microscope. Air-drying has previously been used for different tissues by *Boyde et al.*, (1968), *Hodges*, (1970) and *Luse*, (1970). In this study the same method has been used for the normal intestine and the irradiated intestine. As the specimens spontaneously turned inside-out, the villi were spread making examination easier. Transverse slices of the ileum were also prepared, but here the villi were tightly packed and no meaningful examination could be done. The villi of the ileum, and especially those of the jejunum, are very fragile and it was difficult to find the right dehydration regime suitable to get well preserved villi without any sign of artifacts. The critical-point technique by *Anderson*, (1951) was used for checking normal and irradiated intestine. As scanning electron microscopy (SEM) revealed no differences between the

two techniques, air-drying was chosen as being a more practical method for preparing such a large material. After irradiation, the specimens, probably due to an increased oedema, had to be handled with extreme care in preparation to avoid injuries. As scanning electron microscopy of damaged specimens could be misinterpreted as being some form of irradiation effects, examples have been presented in Chapter VI: Artifacts.

Three air-dried specimens were placed on each aluminium mounting stub (12 mm in diameter) with special adhesive free from injurious chemicals. In order to avoid charging by electrons upon exposure to the electron beam, conductile silver paint was applied to the edges of the specimen. For electron microscopy the specimens were covered by evaporation technique with gold (40%) and paladium (60%) to a thickness of 200 Å during tilting and rotation of the stub. A sputtering method was also tried with 60% gold and 40% paladium, but the results were not as good as those of the evaporation technique. A Cambridge Stereo-Scan Mark II A microscope was used at an accelerating potential of 10–30 kV, and pictures were usually taken at 45° angle. Magnifications from 20× up to 50,000× were possible. While recording on a 2000-line television display tube of the microscope, the specimens were turned and tilted so that they could be examined from all sides, but extreme care had to be taken to get optimal conditions of light, angle, focusing and magnification in each picture. The photographs were recorded on FP3 Ilford Film and enlarged.

Chapter IV

SEM of the normal small intestine



Fig. 4. Villi, high, erected in a regular pattern. Extrusion cells (b) at the top, folds and Goblet cells (a) on the side. 400 $\times$.

Introduction

The inner surface of the ileum changes its appearance due to physiological dynamics. This is reflected in the SEM pictures at their high magnification as a variation between the fasting and the non-fasting animal. In this study the digestive process is therefore an important parameter as far as the effect

of ionizing irradiation is concerned. A reference level or a steady state can be assumed to be obtained in specimens from animals which have been fasting for two days. An investigation of the difference between the two conditions, i.e. the intestine from animals deprived of food and the intestine of normal eating animals, has been considered necessary.



Fig. 5 (a). Enlargement of Fig. 4. Slender, narrow villi with long ridges. Extrusion is going on (a). Deep folds along the side (b). 1000 $\times$.

A. The fasting animal

Villus

Fig. 4 shows a part of the intestine. The villi are thin, leaf-formed, erected and arranged in a regular specific pattern. This technique does not permit measurement of the height of the villi. The length is estimated to be about $200\text{ }\mu\text{m}$ and the thickness to about $20\text{ }\mu\text{m}$. The distance between the midpoint of a villus to the same point on the next villus is about $100\text{ }\mu\text{m}$. The number of villi per square mm is about 180. The folds on the surface are clearly seen running along the villus' side and transverse at the top (Fig. 5 (a)). The openings of small Goblet cells are seen. Cells are seen loosening in the extrusion zone. A comparison of the SEM picture with the ordinary light microscopy technique is shown in Fig. 5 (b), taken from the same animal as in Fig. 4. Villi have a monocellular layer of epithelial cells and Goblet cells coming from the crypts of Lieberkühn, which is visualized by the LM technique, suitable for cell kinetic studies.

Light microscopy and transmission electron microscopy gives the two-dimensional information. With the SEM technique, the villi are seen in a three-dimensional perspective, which gives additional information for interpreting the LM and TEM pictures.

Epithelial cell

The normal villus is covered by a monolayer of columnar epithelial cells intermingled with Goblet cells. In the lower part and on the side of the villus, epithelial cells have a flat surface and a dense connection to each other with slightly marked borderlines. Fig. 10 shows the side wall of a villus with one fold running transversely. At the top of the villus the epithelial cells are more spherical. The area of the cell is difficult to calculate. *Zetterquist* (1956) measured the surface to be $15\text{ }\mu\text{m}^2$. *Marsh*

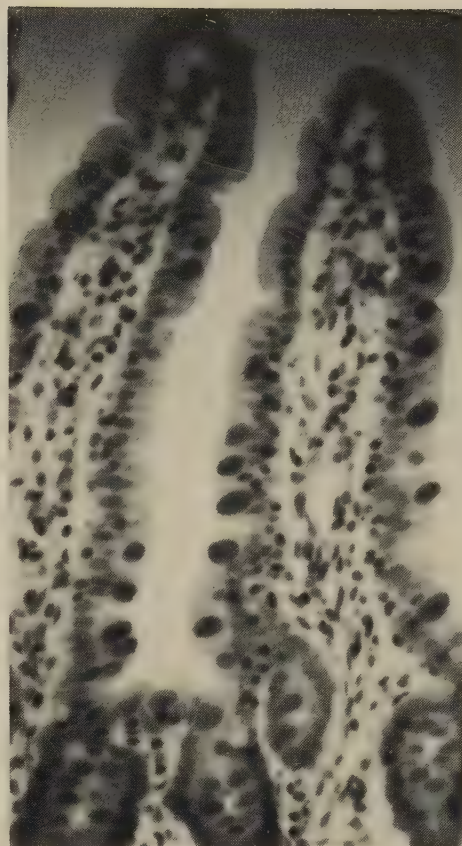


Fig. 5 (b). The same specimen seen in light microscopy. Two villi with deep folds. Dark mucinous content in the Goblet cells. $800\times$.

and *Swift* (1969), using SEM technique, reported the cell area to be between $40\text{--}45\text{ }\mu\text{m}^2$, assuming the cell to be circular with a diameter of $7.2\text{--}7.9\text{ }\mu\text{m}$. *Asworth et. al.* (1963) and *Brown* (1962) considered the area as being 16 and $15\text{ }\mu\text{m}^2$, respectively.

The differences in these reports may be explained by changes in the surface area during the transport of the cell from the bottom to the top of the villus. Fig. 15 shows the borderlines between epithelial cells. In this region the cell area is hexagonal and calculated to be about $16\text{ }\mu\text{m}^2$.

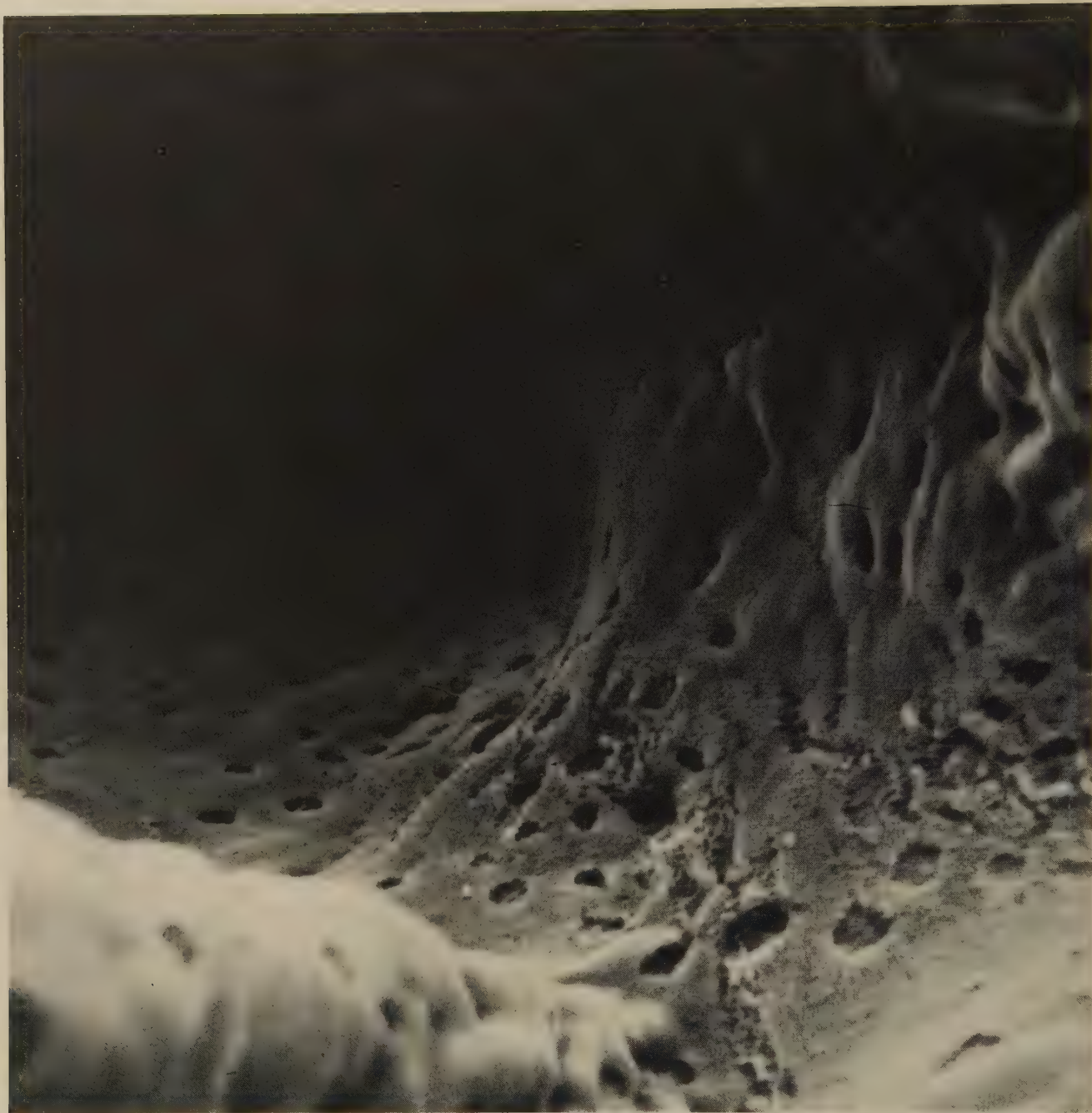


Fig. 6. Bottom between two villi. One side wall seen to the right. Openings in the surface are Goblet cells or crypts. 2000 $\times$.

Microvilli

The epithelial cell surface is covered by a layer of microvilli which forms a carpet of tightly packed rods, arranged in a strictly linear system at an angle of 60° , (Fig. 16). The diameter of the microvilli varies between $0.08 - 0.10 \mu\text{m}$. For comparison the diameter was also measured in a TEM preparation

with the same result. Due to the great variation in the cell area, it is difficult to give a precise number of the microvilli per cell, According to *Torner and Carr* (1968) each cell in various reports has between 600–3000 microvilli. In this investigation the number obtained was about 1600. The number of microvilli per square micron in this study was calculated to be 103 ± 9 , as measured from 20 different areas.

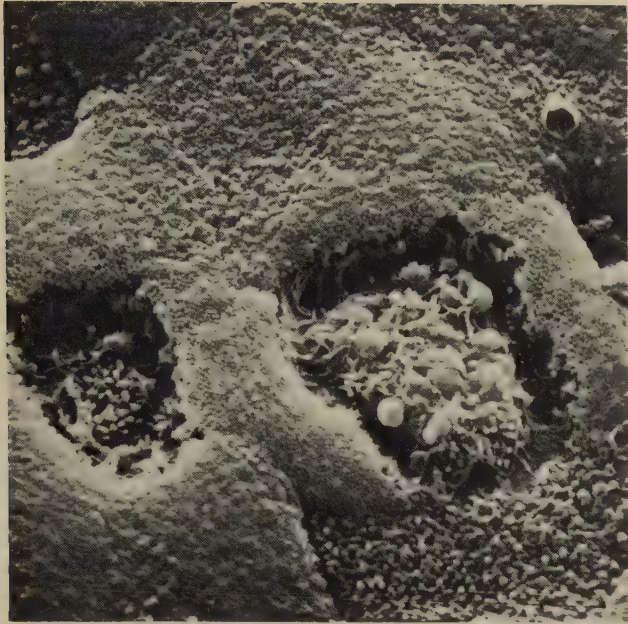


Fig. 7. Two Goblet cells. A few microvilli on the closed membrane to the left. Mucinous granulae penetrating the surface of the right cell. 5000 $\times$.

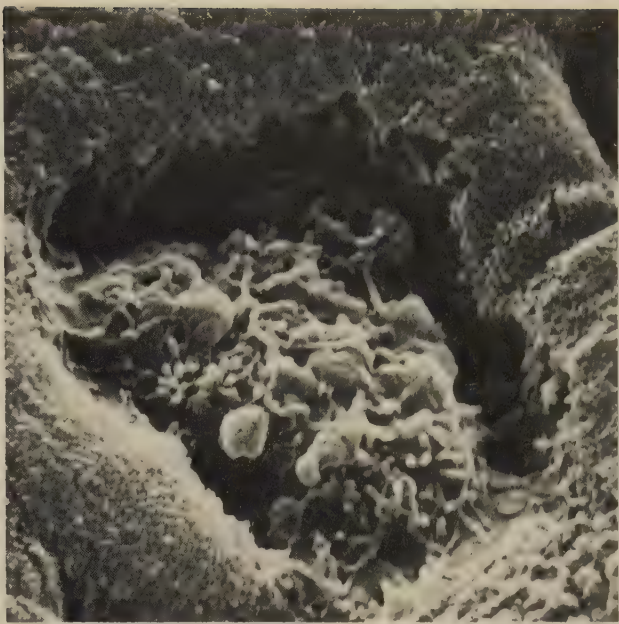


Fig. 8. Enlargement of the cell to the right in Fig. 7. Tightly packed microvilli on the surrounding epithelial cells. 10,000 $\times$.

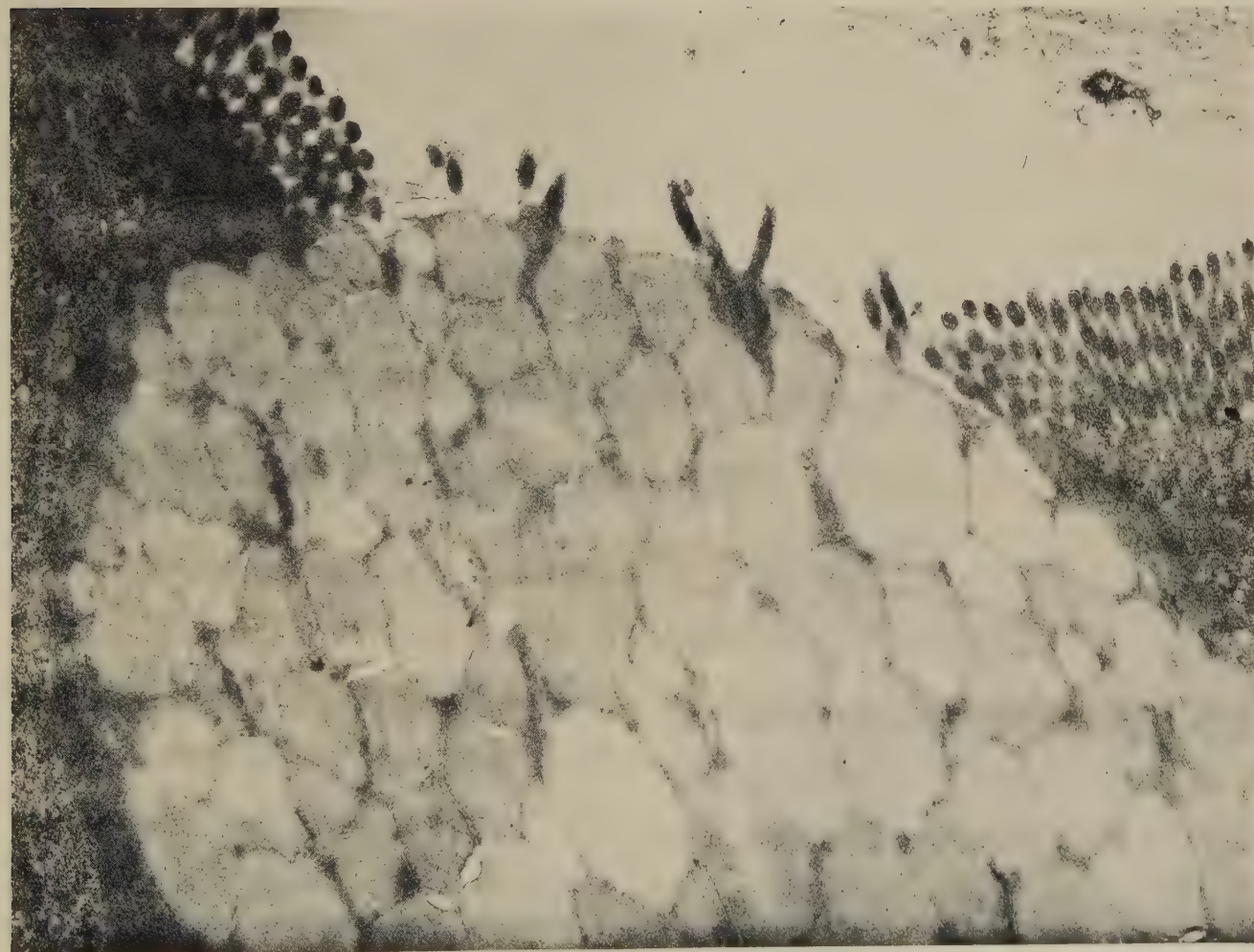


Fig. 9. High magnification of the mucinous granulae of a Goblet cell almost protruding into the intestinal lumen. Note the various size of the packed granulae. 44,000 $\times$. (TEM.)



Fig. 10. Side of a villus. One fold, many Goblet cells and mucinous granulae on the surface. 925 $\times$.

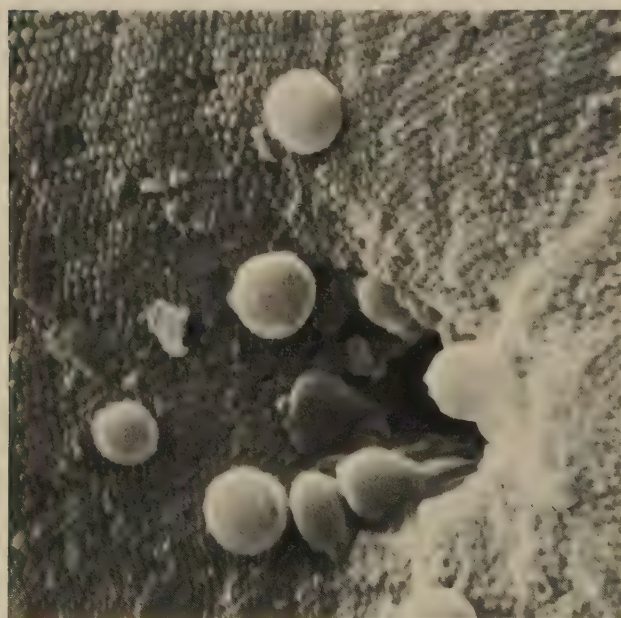


Fig. 11. One of the Goblet cells, Granulae expelled. 9200 $\times$.

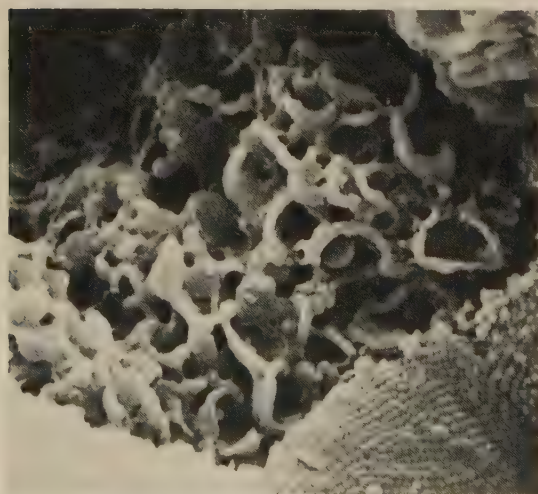


Fig. 13. High magnification of the emptied Goblet cell with the framework left behind. 9200 $\times$.

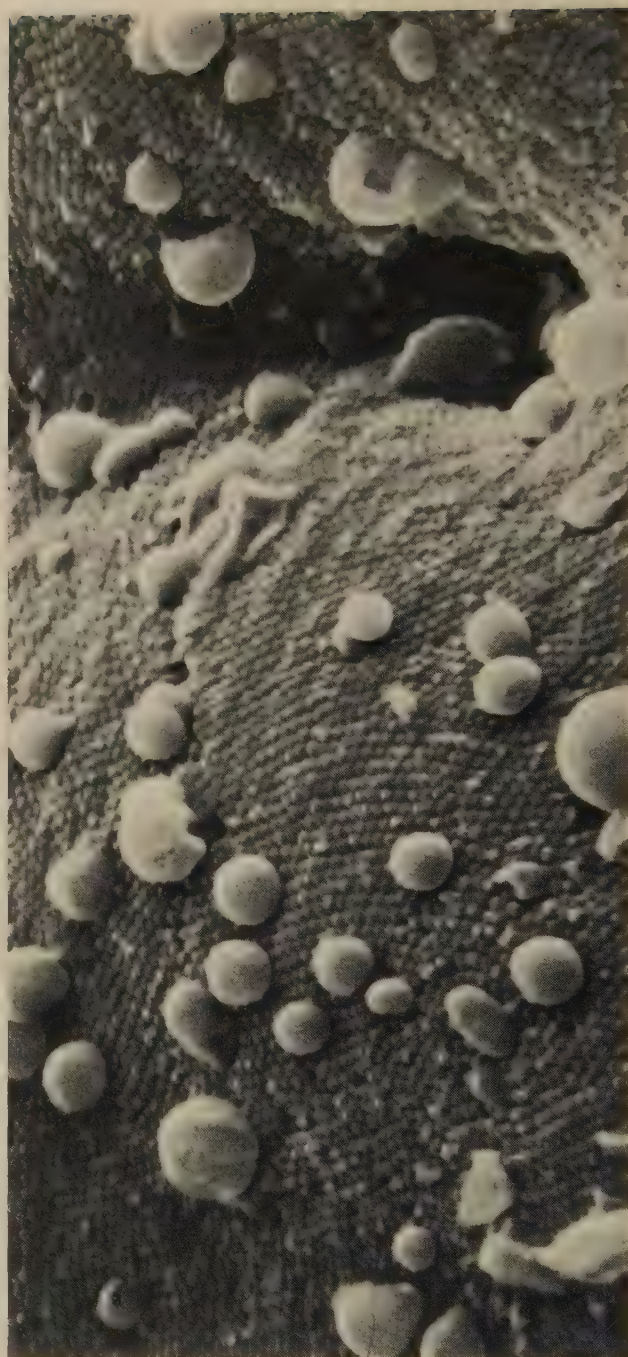


Fig. 12. All the granulae are spread over the smooth microvilli covered epithelial cells. Empty Goblet cell opening in the upper part. 12,000 $\times$.

The goblet cell

The crypts of Liebekühn are the matrix for the Goblet cells. The G-cells move up to the extrusion zone. Their lifetime is 36–48 hours according to *Leblond* and *Stevens* (1948). The G-cell builds up a content of small globuli containing a mucinous product. Having formed secretion globuli, the G-cells seem to wait for a signal to empty their contents. The discharge of secretion may be apocrine according to *Trier* (1964) or merocrine as claimed by *Palay* and *Karlin* (1958). Filled G-cells

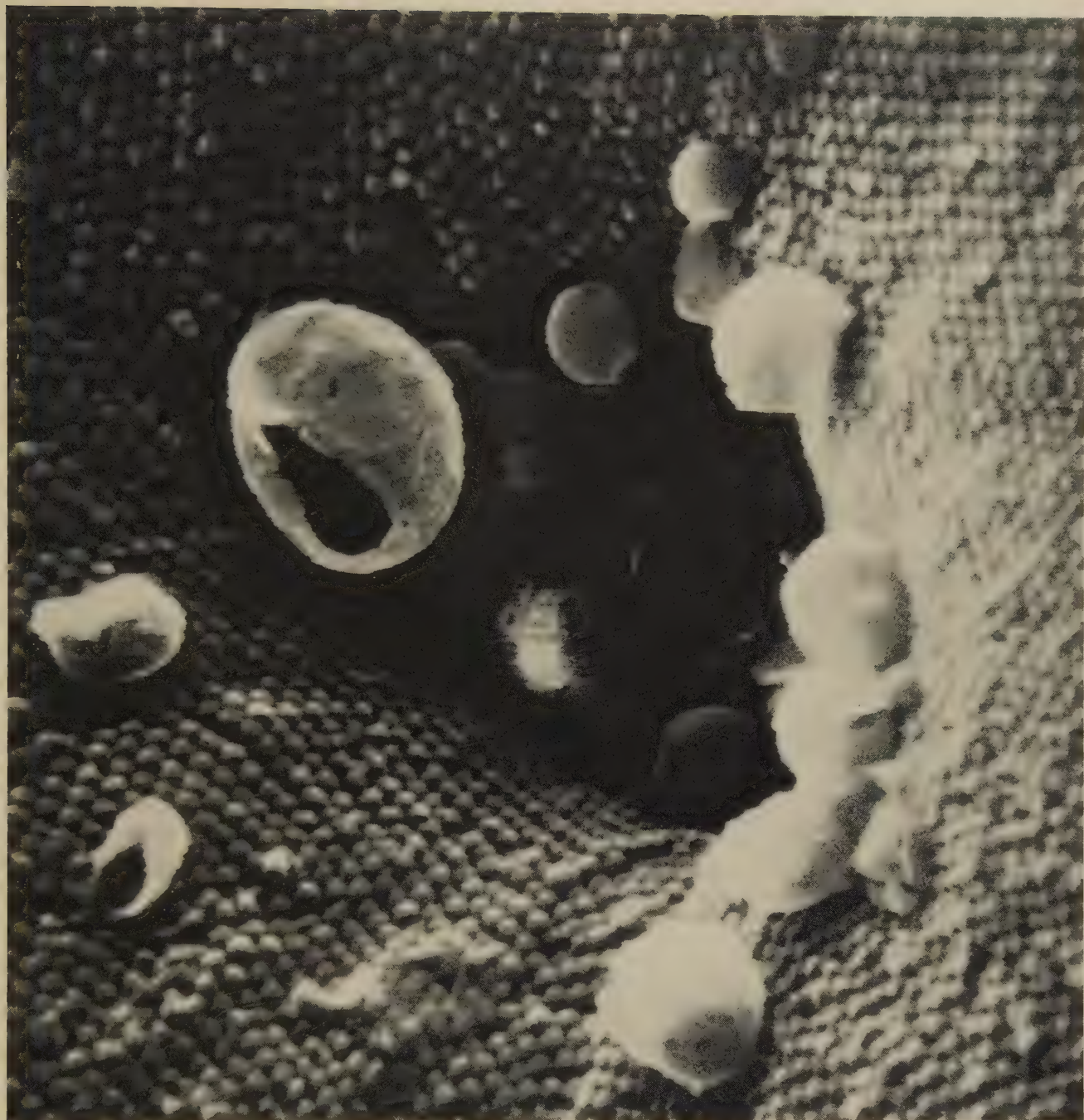


Fig. 14. Secretion of the mucinous granulae. Each granula seems to rupture when passing into the intestinal lumen. 40,000 $\times$.

are found both at the bottom of the villus and at the top (Fig. 5 (b)). Fasting animals do not discharge material from the G-cells. Using SEM technique the openings of the G-cells are clearly seen (Fig. 10). One opening is surrounded by 5–10 epithelial cells. Maximally the openings do not reach a diameter corresponding to the diameter of the cell. Fig. 7 and Fig. 8 show G-cells in the beginning of their phase of discharge. The corresponding appearance with TEM technique is shown in Fig. 9. Each secretion granula is surrounded in the Goblet cell by a membrane.

According to TEM technique the diameter seems to vary widely, which is also demonstrated on SEM. The content is spread out as small balls over the smooth microvilli area of the epithelial cells. Some of the granulae are disrupted and have lost their mucinous substance (Fig. 11 and Fig. 12). The granulae have a diameter of 0.8–1.1 μm , the same as reported by *Krakovic* (1963). The emptying process of the G-cells ends with an opening, showing a framework after the secretion granulae and other remnants of the cell. Whether the Goblet cells are



Fig. 15. The two types of cell on the villus. Goblet cells, empty, and polygonal, plain epithelial cells where the borderline is hardly seen. Perfect microvilli create a smooth surface. 10,000 $\times$.

refilled or not cannot be observed by SEM. As mentioned the Goblet cells in fasting animals move up to the top of the villi closed and probably without being emptied. When the secretion of granulae is completed the empty opening may be closed and no longer visible. Another possibility is refilling and a new membrane against the intestinal lumen. To demonstrate the process of secretion from the

Goblet cells, which does not occur in fasting animals, the rats were given some food and were then sacrificed before the food had reached the ileum. The technique was used to present the process against the clean but otherwise not functioning villus, as seen by SEM. Whether the signal initiating secretion is given locally or from some distance has not been further investigated.

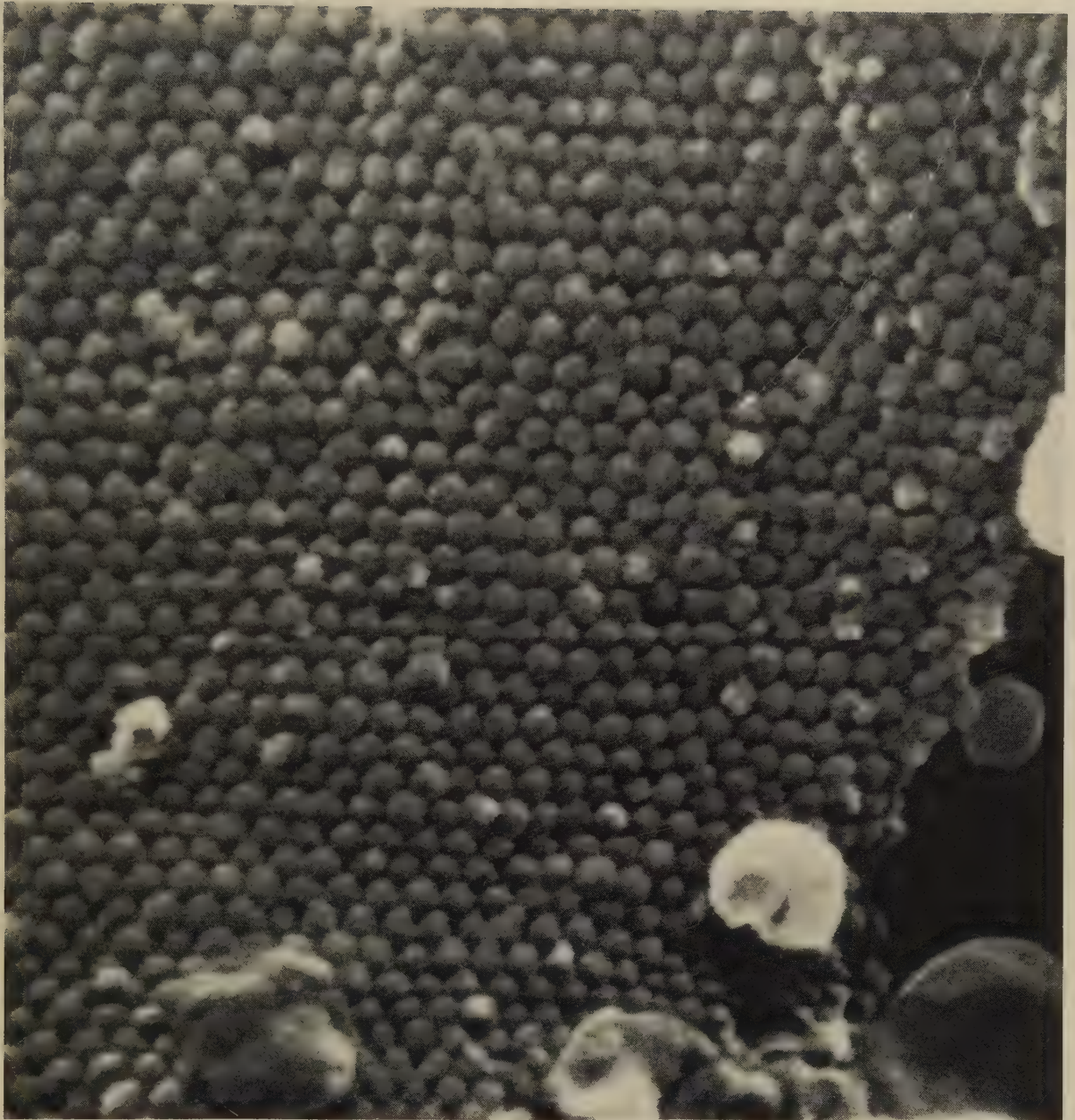


Fig. 16. Part of a cell surface. The arrangement of the microvilli in rows at 60° angle can be seen. The tips of the microvilli are hexagonal or circular. Diameter 0.08 μm . No space between them in fasting animal. 67,000 $\times$.

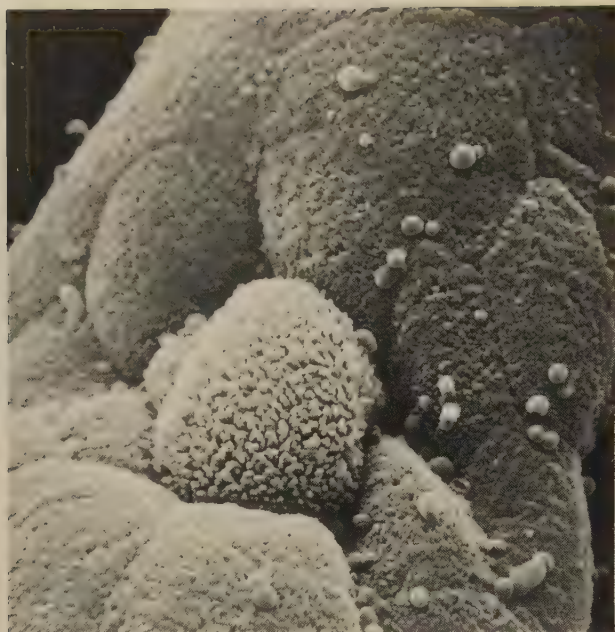


Fig. 17. Process of extrusion. Top of a villus. The cell in the center separates, or becomes spherical on the surface with sparse microvilli. 4700 $\times$.

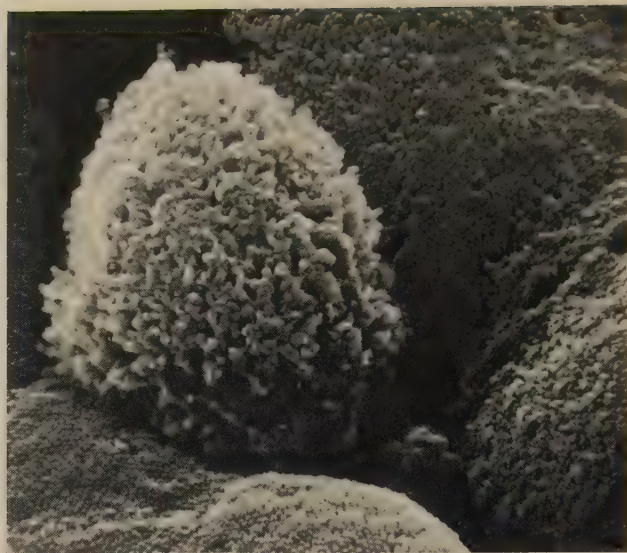


Fig. 18. Process of extrusion. The cell is leaving. Normal microvilli on surrounding cells. 7800 $\times$.

Process of extrusion

The turn-over time is short in the small intestine. The migration of the cells from the crypt bottom to the top of the villus as measured by labelling technique varies in different parts of the intestine and in different conditions, but lies between 36 and 48 hours. In the crypts, new cells are produced in the proliferative compartment.

Higher up in the crypt the cells are maturing but full physiological activity is reached only at the upper third of the villus. Each cell migrate about the breadth of two cells per hour and each villus is served by at least 10 crypts. Every hour 50–100 cells have to leave each villus. In Figs. 17–20 the extrusion process is shown, as seen by SEM. Along the top ridge of each villus one to three different areas of loosening cells can be observed (Fig. 4). It is not known if the cells can be extruded at any location on the top. There are different theories about the migration pathway of the cells. At first the cell separates from the surrounding cells and at the same

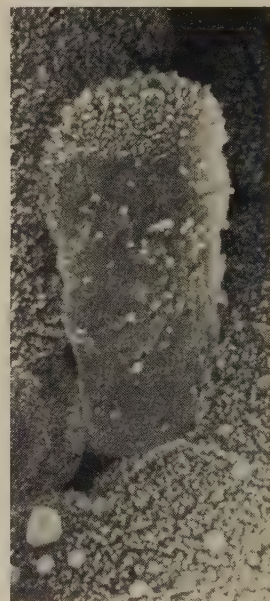


Fig. 19. Process of extrusion. The columnar cell is almost free. 5100 $\times$.

time the surface becomes spherical with microvilli standing sparsely. The high columnar cell then comes out into the intestinal lumen leaving a polygonal opening. The procedure takes only some minutes and is not seen on each villus. The effect of irradiation with increased extrusion activity makes the process important.



Fig. 20. Process of extrusion. Empty hole when the cell has disappeared into the intestinal lumen. The other cells show no evidence of leaving. 19,900 $\times$.



Fig. 21. Peyer's patch. Nine nodules with circular domes towards the intestinal lumen. Villi between them. 100 $\times$.

Peyer's patch

Peyer's patches can be located within the irradiated volume in the lower part of the ileum. In the SEM picture (Fig 21), this lymphatic tissue is seen as 9–15 separated noduli, surrounded by villi and with a

circular, convex surface towards the intestinal lumen. The dome of one nodule (Fig. 22) has a rather smooth surface in the periphery and microridges converging towards the center, where there seems to be an extrusion zone with cells in different degrees of extrusion. In this area (Fig. 23) epithelial

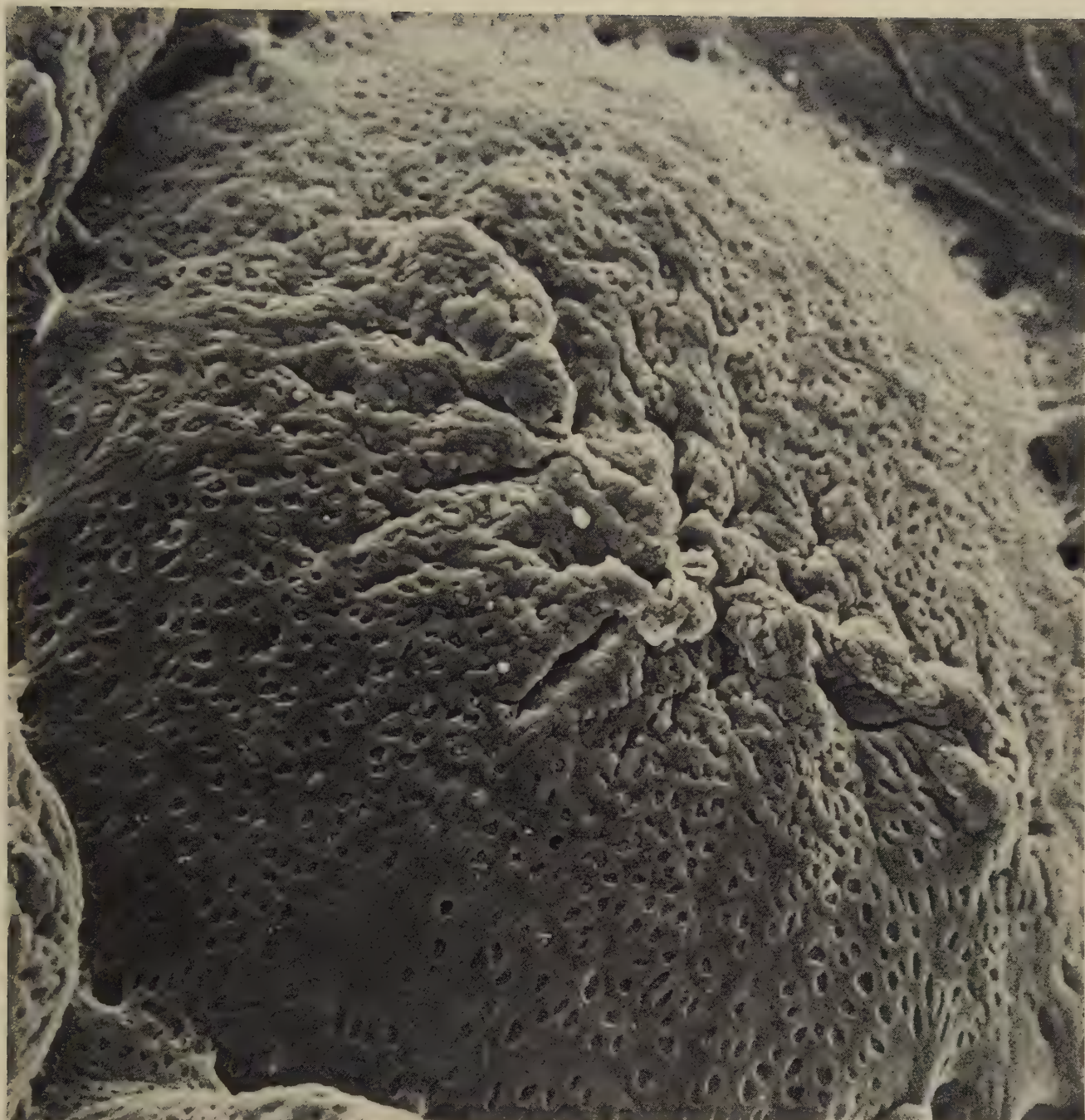


Fig. 22. One nodule with convex surface. Ridges converging to the center. M-cells all over the surface. 500 ×.



Fig. 23. The center of Fig. 22. Ridges with cells with half spherical surface. Extrusion in the center 2000 $\times$.

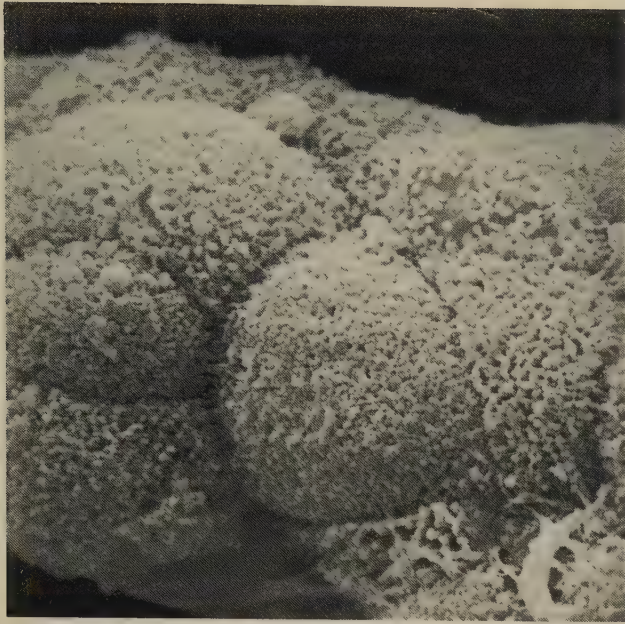


Fig. 24. Enlargement of one ridge. The cells are swollen, take different forms and have sparse microvilli. 4680 $\times$.

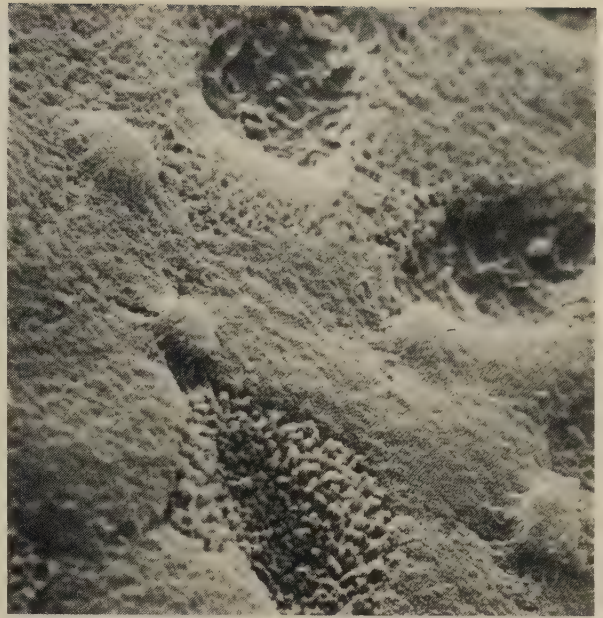


Fig. 26. Microvilli-covered cells. Two M-cells. At four points, intercellularly, there is a raising of the surface. Lymphoid cells? 4680 $\times$.



Fig. 25. The periphery of a dome with part of a villus and with M-cells on the surface. 600 $\times$.

cells have a convex surface on the ridges with diverging microvilli (Fig. 24). In the periphery on the other hand, epithelial cells are flat with tightly packed microvilli with the same diameter as on the villus cell. In the peripheral zone (Fig. 25) the surface is dominated by "holes", which at a high magnification are shown to be concave surfaces containing only a few, sparsely spread microvilli-like structures. These cells are known as "M-cells" (Owen and Jones 1974). (Fig. 27). What appear to be microvilli are called microfolds. The striking resemblance between these cell surfaces and those of unemptied Goblet cells is obvious. In addition, small shallow protrusions are seen on the peripheral surface, always at the borderline between the epithelial cells (Fig. 26). The nature of this finding has not been clarified by means of SEM or by LM technique in this study. It has, however, been proposed that lymphoid cells migrate to the surface of the intestine between the epithelial cells in the villi and may be also in the Peyer's patch.



Fig. 27. M-cells. Isolated microvilli-like structures on the surface.
10,000 $\times$.

B. Non fasting animal

In this natural condition where the rats have free access to water and commercial pellets, the working mucosa of the villi in the ileum is different from that found in fasting animals. Instead of high, erected, clean villi in a regular pattern a picture of retracted

villi with increased marking of folds and an increased extrusion process is seen by SEM. The villi appear to be tightly packed because of the retraction. The folds are accentuated even in the vertical direction. The thickness is about $40\ \mu\text{m}$ and the length $250\ \mu\text{m}$. The distance between the midpoints of villi in the same row is about $100\ \mu\text{m}$. (Fig. 28).



Fig. 28. Non fasting animal. Retracted villi. Deep folds. Bacteria on the upper third. $400\times$.



Fig. 29. Broad base of the villi. Bottom cannot be seen. Bacteria. 470 $\times$.



Fig. 30. Cells in extrusion. Penetrating bacteria. 1000 $\times$.

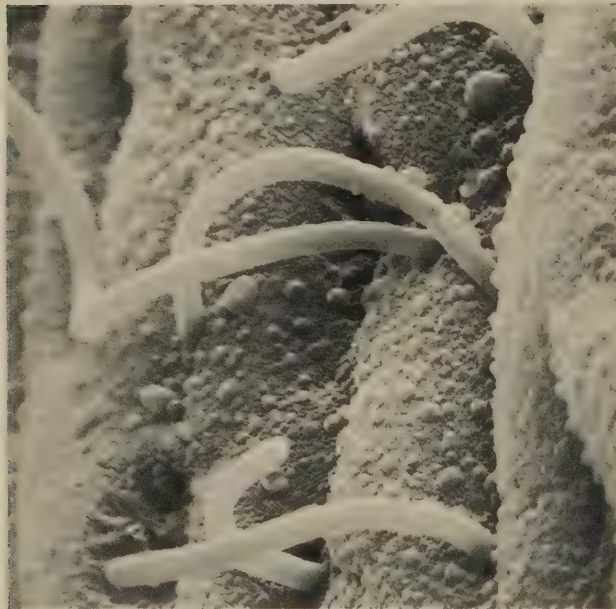


Fig. 31. Side of a villus. Mucinous granulae and bacteria. Tightly packed microvilli. 4680 $\times$.

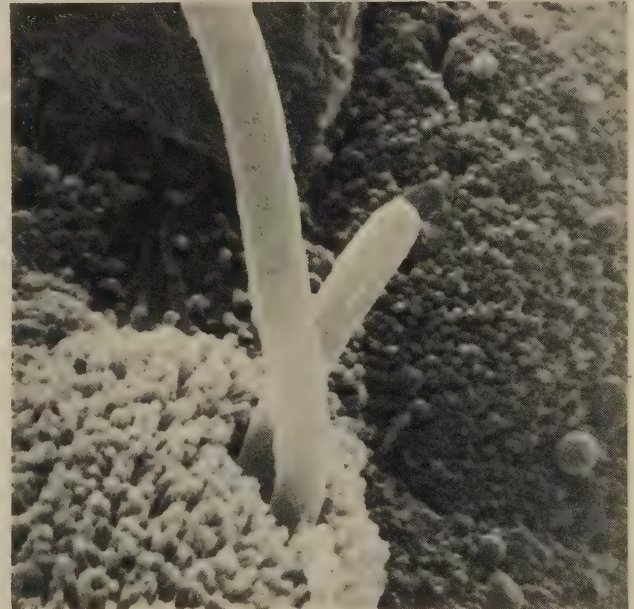


Fig. 32. Enlargement of Fig. 30. Two bacteria penetrating the vertex of an extruding cell. 9960 $\times$.



Fig. 33. Lumen between two villi. Lots of bacteria. 1870 $\times$.



Fig. 34. Multiple attack by bacteria on the upper part of the villus. 4680 $\times$.

The epithelial cells have a more convex surface and the microvilli do not form a tight carpet as in fasting animals, but are now more sparse. The most striking difference is the existence of bacteria attacking and penetrating the epithelial cells on the tops and the upper parts. They have been classified as fusiforme anaerobe bacteria, and the penetration is regarded as a physiological condition (see "Bacteria" in this chapter). On the surface, mucinous granulae are spread, showing the result of Goblet

cell activity in non fasting animals. In the Peyer's patch, the calm surface is replaced by signs of activity in the central section, where an increased extrusion is going on and bacteria are attacking. In the peripheral area, cells are seen to penetrate the surface. This action seems to occur between the epithelial cells and also through the concave surface of the M-cells. No bacteria attack any peripheral part of the Peyer's patch.

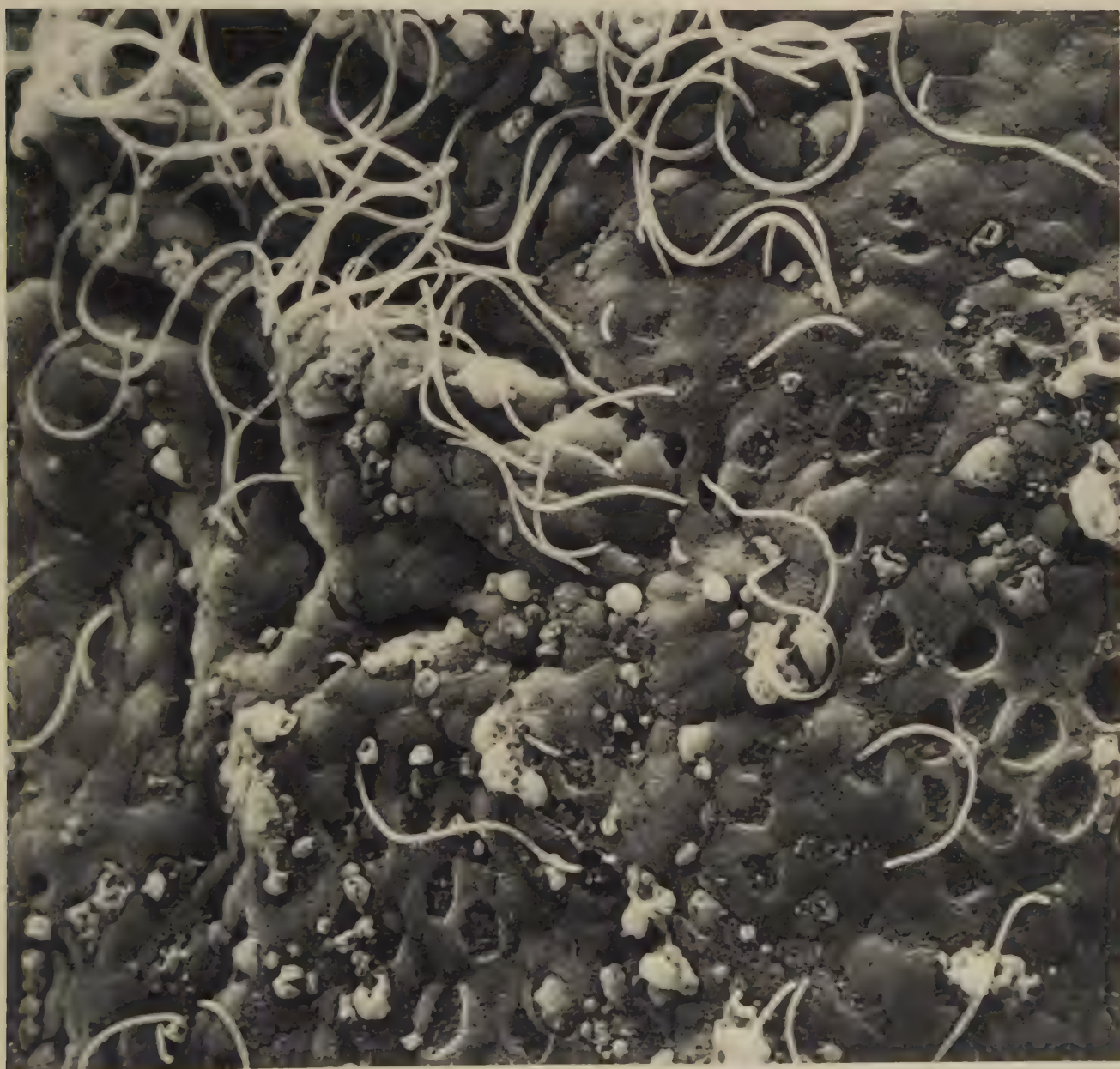


Fig. 35. Normal microvilli on the convex epithelial cell surface.
Three bacteria penetrating between the cells. 21,500 $\times$.

Fig. 36. Peyer's patch. Central area in non fasting animal. Bacteria. 470 $\times$.



Fig. 37. Non fasting animal. Surface of the Peyer's patch. Bacteria. Cells coming through the surface. Many closed M-cells. 1000 $\times$.



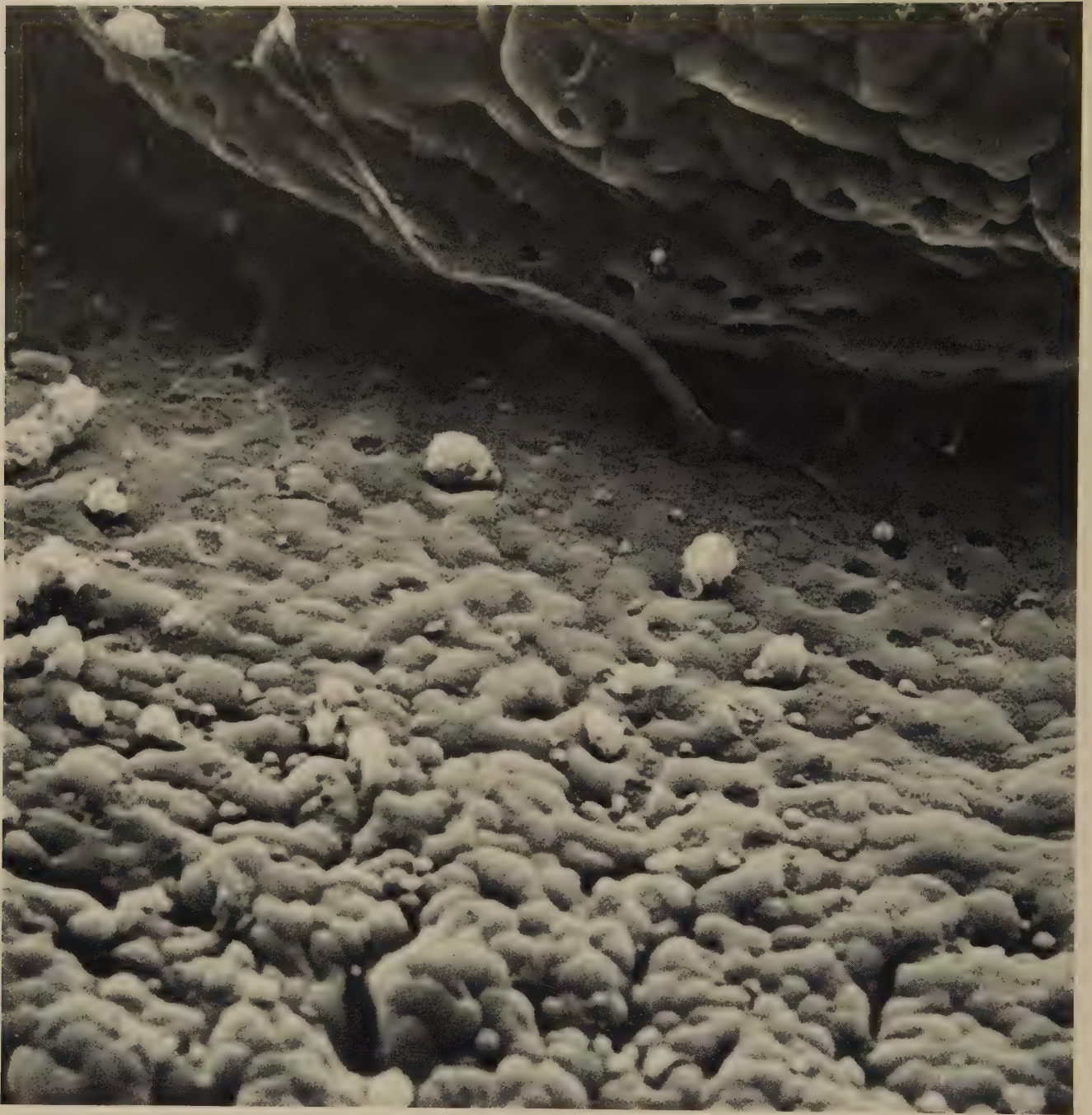


Fig. 38. Non fasting animal. Peripheral part of the Peyer's patch. Wall of a villus at the upper part. Cells are coming through some of the M-cells. 1000 $\times$.



Fig. 39. Enlargement of Fig. 38. 4890 ×.

C. Bacteria

The villus normally functions side by side with a rich flora of bacteria, forming an important ecological component of the intestine. Little is known about the biochemical mechanisms between bacteria and the mucosal membranes. Many organisms attach to the epithelium through specific surface-to-surface interactions. Particular bacterial types attach selectively to certain epithelium. In rats and mice (Savage, 1969) from certain conventional colonies, the villous epithelium in the ileum is covered by long chains of rods or cocci. Bacterial cells of similar

morphology have been seen in preparations examined in the electron microscope (Hampton and Rosario 1965).

The large bowel has anaerobic fusiform bacteria that form colonies in the mucus and attach to the mucosal epithelium (Savage *et. al.*, 1971). Earlier studies, mostly by light microscopy, did not clearly demonstrate this contact to the cell membranes, but could verify a lot of fusiform bacteria in the fuzzy coat lying in the mucosal cells (Nelson and Mata, 1970).

Particular surface properties of bacterial cells undoubtedly mediate their attachment to mucosal



Fig. 40. Non fasting animal. Retracted villi .Bacteria. 1000 $\times$.

epithelia (Savage, 1972). In the rodent stomach, indigenous lactobacilli attach to keratinized squamous cells and to each other through a substance on their surface that may be a mucopolysaccharide (Savage *et al.*, 1968; Takeuchi and Zeller, 1972). The physiological state of epithelial cells may also influence the attachment and penetration of bacteria.

In the small intestine the columnar cells migrate up along the surface to the top of the villi and are extruded. As this migration is taking place, the cells undergo physiological changes detectable as alterations in the activity of certain enzymes. Cells

nearest the top have the highest activities of dipeptidases, disaccharidases and alkaline phosphatases (Nordström *et al.*, 1967). If the rates of migration of the cells are changed, the activities of these enzymes would also be affected in some way.

Enzymes like disaccharidases are involved in membrane function on the luminal border of intestinal epithelial cells (Moog and Grey, 1968). Changes in the activities of these enzymes may alter the physiology of the membrane and the ability of the cells to accept attaching bacteria. In some intestinal infections the bacterial pathogens attach more readily to epithelia near the villous top than at



Fig. 41. Non-fasting animal. The same specimen as in Fig. 40. After treatment with mannose all bacteria are gone. 1000 $\times$.

the bases (*Drees and Waxler, 1970*). In other infections the opposite is true. Therefore the attachment of organisms may in part at least be determined by the physiological state of the cell membrane (*Savage, 1972*).

The fusiform bacteria may be anaerobes, in large concentrations (10^{10} – 10^{11}) per gram in caecum) and forming a group that has achieved a symbiotic relationship with their host, having invasive properties (*Dubos et al., 1963; Hughes, 1972*). There have been difficulties in culturing these bacteria, which require careful anaerobic conditions and are very sensitive to O_2 . They die after 20–30 minutes in air (*Lee et al., 1969*).

Scanning electron microscopy of bacteria has demonstrated a lot of different types of micro-organisms (*Klainer and Perkins, 1971; Whittaker and Drucker, 1970; Drucker and Whittaker, 1971*). *Takeuchi and Zeller, 1972*, presented spirochete-infested colonic mucosa of the rhesus monkey, reporting the so far rather limited observations of pathological conditions as a host-parasitic relationship.

Results from a comparison between normal and germ-free rats (*Abrams et al., 1963*), show that the rate of loss of cells from the extrusion zones is accelerated by the existence of bacteria.

Erlandsen and Chase, 1974, found a heavy colonization of segmented filamentous bacteria in the ileum of the rat most abundant on the top and on the upper third of the intestinal villi. No visible change could be seen on the epithelial cells at SEM but in heavy attack it was no longer possible to discern any recognizable features of the microvillous border, suggesting that the cells did not seem to be able to function normally. The penetration was superficial, as recorded by TEM. Despite this change in microvilli, *Erlandsen and Chase, 1974*, suggested that the microbe and the host may coexist in at least mutual tolerance.

Indirect evidence points to the possible contractility of microvilli and together with the presence of actin-like filaments capable of binding heavy meromyosin it is assumed that filaments in the attachment sites may contribute to adherence by some form of contractile mechanism (*Ishikawa et al., 1969*).

On the other hand, *Erlandsen and Chase (1974)* refer to a first line of defense on the microvillous border where secretory immunoglobulins and lysozymes are reported to be absorbed by the luminal surface coat of intestinal epithelial cells, and these in combination with complement have been shown to provide a potent bactericidal system in vitro (*Adinolfi et al., 1966; Porter, 1972*).

According to *Erlandsen–Chase (1974)*, there

exists a cyclic release and reattachment of new holdfasts in spite of continual epithelial turnover.

In this SEM study with female Sprague and Dawley rats, the same type of bacteria, segmented, filamentous and fusiform, was found to be attached to the upper third of the villi, but only in non-fasting animals, which means animals having free access to water and pellets of a commercial type. On the other hand, it was not possible to demonstrate any fusiform bacteria in fasting animals, which means rats on a water diet.

Specimens were always taken from the lowest part of the ileum. The colonization of bacteria does not change much between individually non-fasting animals. The impression was that when the special part of the ileum, taken for examination, was empty from any notable mass of content, the number of bacteria was low.

As has also been mentioned by *Erlandsen–Chase (1974)*, in this study no morphological difference was seen at SEM between the attached cells and the cells free from bacteria. On the other hand there was never such a large attack that the microvilli covering the surface of the epithelial cells were destroyed (*Fig. 34*).

The difference between fasting and non-fasting animals was also observed in the Peyer's-patches, where the central part in non-fasting animals was more or less attacked by fusiform bacteria while the peripheral part did not have any bacteria (*Fig. 38*).

The relation of bacteria to irradiation is presented in Chapter VII and Chapter VIII.

The surface coat or enteric coat on the microvilli is a sulphated, weakly acidic mucopolysaccharide-layer where the disaccharides are hydrolyzed. Precursors to the enzymes are among others mannose and galactose as found by 3H -thymidine technique (*Ito, 1965*). The transport from the Golgi-apparatus of the epithelial cell to the enteric surface takes 30–60 minutes (*Ito, 1965; Greenberger, 1969*). The surface coat is fixated by glutaraldehyde and difficult to remove, and is different from the mucus that is emptied into the intestinal lumen from the Goblet-cells. The coat changes in species and is rather thin in rat and mouse (*Ito, 1965*).

As suggested by *Savage (1972)* the attachment of bacteria could be in a way determined by the physiological state of the cell membrane. Influenced by this theory and by the finding that bacteria did not attack the mucous cell layer in fasting animals, it was in this study found worthwhile to try the technique published by *Ofek et al. (1977)*. They found that *Escherichia coli*-attachment to epithelial cells in vitro was mediated by mannose- (or mannose-like) receptors present on the surface of the cell. The conclusion was that mannose, a sugar

found on most mammalian cell surfaces, acts as a receptor for binding of *E. coli*, suggesting an approach to the elucidation of the mechanism of bacterial adherence. Saturation of binding sites on the bacterial surface by sugar prevented in their study the attachment of these organisms to the epithelial membrane receptor, containing mannose or a mannose-like structure. Washing with a solution containing mannose (6 or 12 mg ml⁻¹) resulted in the removal of pre-attached bacteria.

To find out if the same mechanism was connected to the attachment of fusiform bacteria in eating animals in this study the following saccharides were tested; fructose, glucose, galactose and mannose. Half of the specimens from each animal was prepared as usual for SEM, and the other half was washed in different saccharides (6–12 mg ml⁻¹) four

times and after new washing the ordinary preparation for SEM went on. The result was the same as reported by Ofek *et al.*, (1977). In non-irradiated eating rats almost all fusiform bacteria disappeared after d-mannose-treatment alone. Fig. 40 and Fig. 41 show specimens from the same animal before and after mannose-treatment.

The theory of a relation between the physiological status of the epithelial cells and the degree of attachment of bacteria is strengthened by this finding of no bacteria in fasting animals and no bacteria after treatment with mannose, a precursor of the surface coat where an increased activity could be initiated by the content in the intestinal lumen. The same experiments were repeated in connection to irradiation with single dose and fractionated doses with or without a state of fasting.

Chapter V

Artifacts

In the 1960's, as soon as transmission electron microscopy (TEM) was more generally known, warnings were heard that artifacts could appear during preparation. In the 1970's the same problem arose in preparation for scanning electron microscopy (SEM). This technique gives a new approach to morphology in three dimensions, often in aesthetically pleasing pictures. *Clark and Glagov* (1976), mean that "applied to biological materials appropriately and critically, SEM may reveal important unanticipated morphological details and relationships", but publish examples which show that artifacts have been presented as new findings. This has, for example, occurred in specimens of the walls of blood vessels if the intraluminal pressure had not been maintained during fixation. Artifacts can appear regularly and are thus considered to be normal structures, while they are in reality either an accretion of extraneous material, distortion of real cell and tissue surface details or optical distortions during microscopy. It is necessary that repeated experiments be done on new findings, using light microscopy (LM) or TEM as a control. Fixation and dehydration of soft tissue for SEM can give shrinking artifacts in the form of small alterations of the surface structure, for instance in using freeze-drying technique (*Pfeiffer, 1970, 1971*). At SEM of biological surfaces, injuries to the specimen or groove artifacts can be observed in the stereomicroscope, which also can be used for control of the metal layer (*Ludwig et. al., 1972*). Dehydration of soft tissues, such as the small intestine, is a delicate and important process. Incubation in vitro of epithelial cells from the small intestine reveals that a rapid swelling occurs within 30 minutes after inhibition of the normal blood flow (*Plattner et al., 1970*).

Critical-point drying with liquid CO₂ (*Anderson, 1951*) or freon (*Cohen et al., 1968*) is considered the best method for avoiding distortion by high surface tension forces of air-drying and ice-damage in freeze-drying (*Meller et al., 1973*). In working out the suitable preparation for SEM in this study, air-drying was compared to critical-point drying using CO₂. The air-drying technique was used adapted to the circumstances. In a fasting rat and in a rat given an oedema-producing dose, fixation in 2% glutaraldehyde for 2 hours was sufficient. Post-fixation in OsO₄ was not used. The procedure for dehydration in acetone was corrected to a longer series of dilutions and longer periods in each bath. In samples with oedematous tissue the stereomicroscope could otherwise during air-drying reveal a kind of explosive reaction giving different degrees of damage to the villi. The whole top could break off during microscopy (Fig. 42), sometimes with isolated single epithelial cells attached to the core (Fig. 43) and Fig. 44), showing the strength of the surface tension forces. The naked top of the villi shows in Fig. 42 how epithelial cells are built up like bricks, but that the core has been isolated, probably due to a discrepancy in the process of dehydration. Such an artifact was observed in this study before a correction of preparations was made at oedema after a single dose of 10–20 Gy, on specimens taken after 24–28 hours or after a fractionated dose of 20 Gy 24–48 hours later. All the epithelial cells covering the villi and the bottom between the villi disappeared and could leave clusters of cells over the crypt openings (Fig. 45 and Fig. 46) or a totally naked intestinal surface lacking epithelial cells, where only the cores of the villi remain after the shedding of all cells and where the crypt openings gape and can be



Fig. 42. Villus without top. Artifact probably due to a difference of the dehydration effect on the epithelial cell lining and the central core resulting in such a tension force that the villus was explosively disrupted, which could be followed in the stereomicroscope. 1650 $\times$.

counted around each villus (Fig. 48 and Fig. 49). The picture agrees with that seen at SEM after autolysis (Toner *et al.*, 1970). Artifacts arising in this manner reveal, however, certain morphological details at the same time, for example on the "wall" of epithelial cells, how the crypt cells are stored when moving up to the functional compartment on the villi from the crypt (Fig. 45, 46, 50) and how the crypts are surrounding a villus.

The naked surface of the core also shows, at high magnification, the capillary system with endothelial cells (Fig. 51, arrow).

It has to be emphasized that these examples of artifacts are different from the effects following irradiation, where there were gradually increasing changes up to denudation. The reason for the difficulties in preparation has to be incomplete dehydration in cases with marked oedema. Correct-



Fig. 43. Villus preparation damage with contact between the core and the cells. 200 $\times$.

ing the preparation technique enabled an avoiding of artifacts, even where there was marked oedema. Even preparation for LM is difficult when oedema is present. An especially careful bedding in plastic had to be tested, as well as a special cutting technique. It is thus necessary to ascertain the right preparation-technique for each situation for the registration of pathophysiological and morphological studies by means of SEM, and also to repeatedly control the results and to test other techniques.



Fig. 44. Higher magnification of Fig. 43, where a few isolated cells are in contact with the irregular surface of the core. 1000 $\times$.

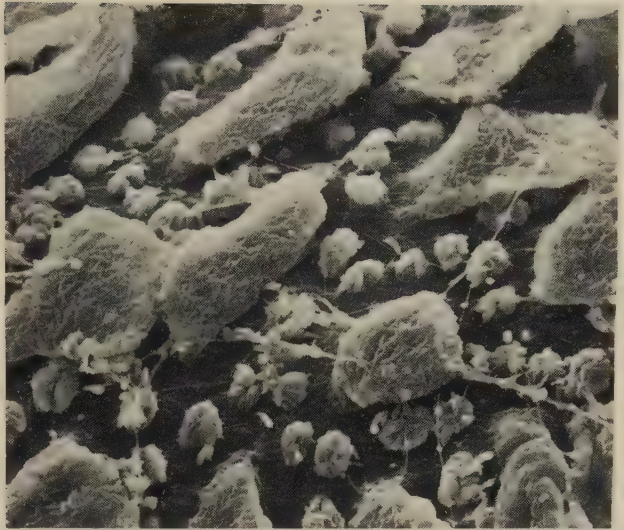


Fig. 45. Extreme artifact. Naked cores are surrounded by small clusters of cells corresponding to the crypt openings, about 10–12 around each villus. Preparation artifact due to irradiation oedema. 100 $\times$.



Fig. 46. Magnification of Fig. 45. The arrangement of the cells in a circle over each crypt corresponds to the picture of a cross section with LM technique shown in Fig. 47. 925 $\times$.

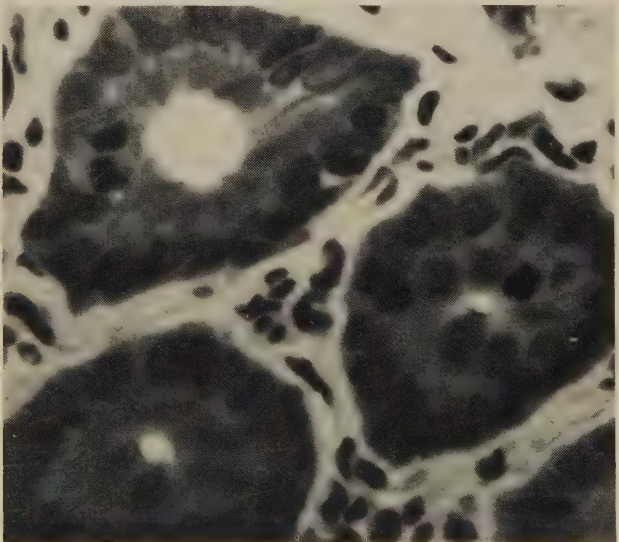


Fig. 47. Light microscopy of crypts in cross section. 900 $\times$.

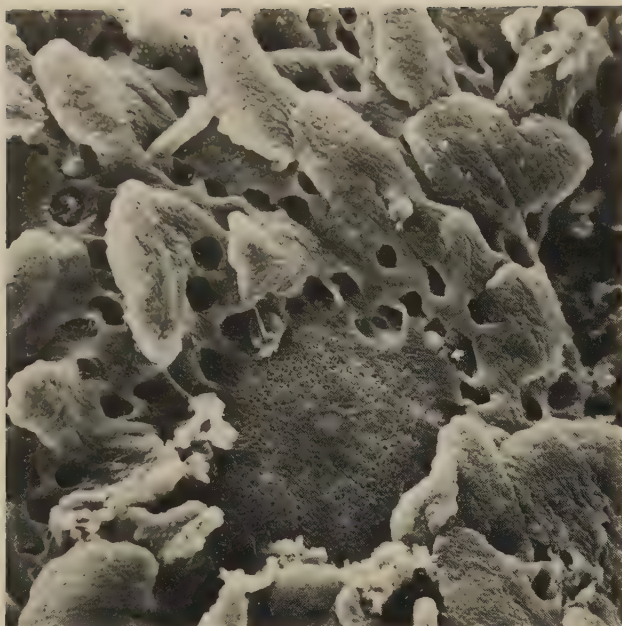


Fig. 48. Result of total shedding of cells. Peyer's patch in the center surrounded by naked cores and empty crypt openings. 100 $\times$.



Fig. 49. Higher magnification of Fig. 48 with empty crypt openings. 450 $\times$.

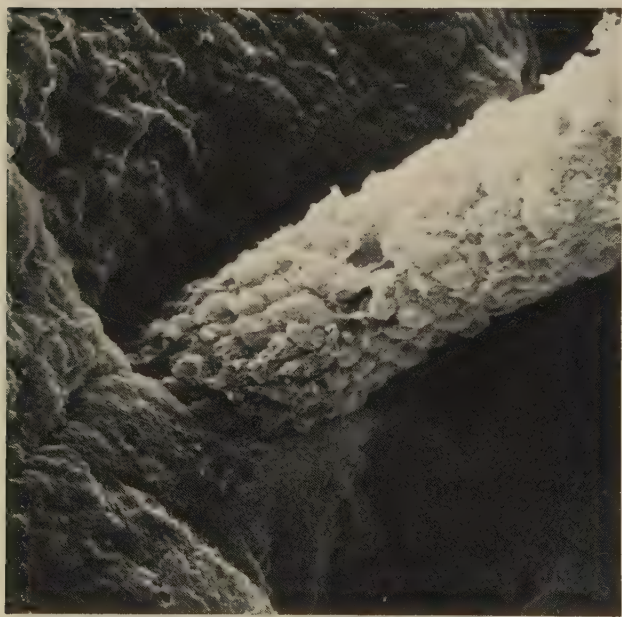


Fig. 50. Crypt cells moving up together like a rod. See also the arrow in Fig. 48. 925 $\times$.

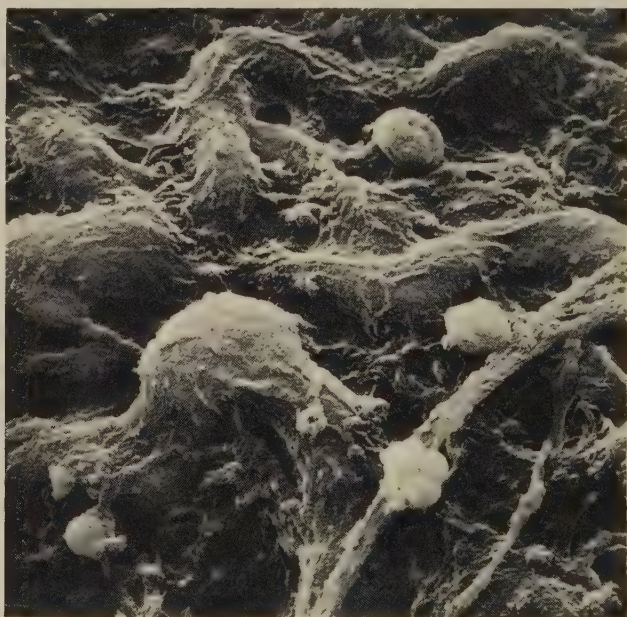


Fig. 51. The surface of a naked core free of epithelial cells with capillaries and single endothelial cells (arrow) bulging up on the surface. 4600 $\times$.

Chapter VI

The score system

Evaluating the effects of irradiation

The effects of irradiation on living matter may be expressed in terms of the survival or death of cells, changes in their reproductive capacity, alteration of the physiological activity of the cells and of their morphological structure. The difficulties in finding objective methods for evaluating these effects are great and many uncertainties are introduced by the different parameters that must be considered.

The irradiation of single cells in vitro as described by *Lea* (1956) and *Puck and Marcus* (1956), has given the basis for an objective study of survival curves. In vivo measurement by the “dilution assay technique” (*Hewitt and Wilson*, 1959) is another example of measuring a surviving fraction of cells in tumor radiation biology. The “spleen colony assay system” introduced by *McCulloch and Till* (1962) is also a “surviving fraction”-system which seems to be capable of making an objective correlation between the irradiation of cells and the effects.

The skin is a suitable object for measuring the effects of irradiation in both animals (pigs) and human beings. *Strandquist* (1944) was the first to employ the human skin for radiobiological investigations. In spite of the fact that many parameters contribute to form the total biological effect of irradiation he was able to construct a time-dose relationship curve which is still useful. *Strandquist's* score system was totally subjective. He divided the reactions into four grades: 1. erythema, 2. epidermitis sicca, 3. epidermitis exsudativa and 4. necrosis of the skin. *Fowler and Stern* (1960) have studied the

effects on pig skin measured in different degrees of damage for analysis of the radiation effects. Later on, similar score systems have been used in studies of time- and dose-relationships (*Berry et al.*, 1974; *Turesson and Notter*, 1975). The procedure has been regarded suitable for both single doses and fractionation studies.

For the small bowel, other units or methods are used for analysing the radiation effects, as the crypt counting method, *Withers and Elkind* (1970) and the cell counting method (*Devik*, 1971). This technique may be assumed to be relevant for the radiation injuries to the intestine. The crypts of Lieberkühn and the villi are so far to be considered an anatomical entity, as the proliferation of cells is going on within the limits created by the physiological activity of the intestine. The villus as a whole is composed of different anatomical sub-units and each cell compartment has its own radiosensitivity. The influence of irradiation is thus a complex situation to investigate.

In the study of the intestinal surface and the effects of irradiation, SEM has proven to be of great value (*Anderson and Withers* 1973; *Carr and Toner* 1972; *Hamlet et al.* 1976; *Carr et al.* 1979). Due to higher power of resolution, and presenting a three-dimensional picture, SEM is able to give information about the villi, epithelial cells and microvilli as well as physiological processes such as the extrusion of cells, the emptying of Goblet cells and the attack by bacteria. As expressed by *Hamlet et al.*, 1976, mucosal morphology by SEM is the total expression of many biological parameters, where regenerative ability of the crypt is only one of them.

The score system

In evaluating the effects of irradiation on the morphology, the results are described as scores in an arbitrary scale. In the scale used, 0.0 shows the normal morphology and 4.0 the greatest injury: ulceration. The score is given as the average reaction in more than two experiments judged by three persons. In an attempt to refine the classification of the effect and to obtain a gliding scale on the score number the SEM pictures were judged with

consideration taken to physiological phenomena seen by this technique on the mucosa.

Villus, epithelial cell and the microvillus were judged separately. The score number of the average reaction is related to a definition of each half step from 0.0 to 4.0.

Score curves have been drawn for each of these three structures where the average reaction is related to time after irradiation, Fig. 186–189.

On the following pages definitions of the score numbers in the three different structures are declared together with examples by SEM.

Villus

- 0.0 Normal villus as described in Chapter IV.
- 0.5 Swollen villus with height intact.
- 1.0 Pyramidal form with shallow folds and with 80%–100% of normal height intact.
- 1.5 Bulbous form with almost no folds and a rather flat surface on the top. Height reduced by 50%.
- 2.0 Ellipsoid form, no folds, only 25% of normal height.
- 2.5 Very short villi, flat surface, ellipsoid form.
- 3.0 Only slight ridges indicating villi.
- 3.5 Flat surface without villi.
- 4.0 Ulceration.



Fig. 52. Score 1.0 90 ×

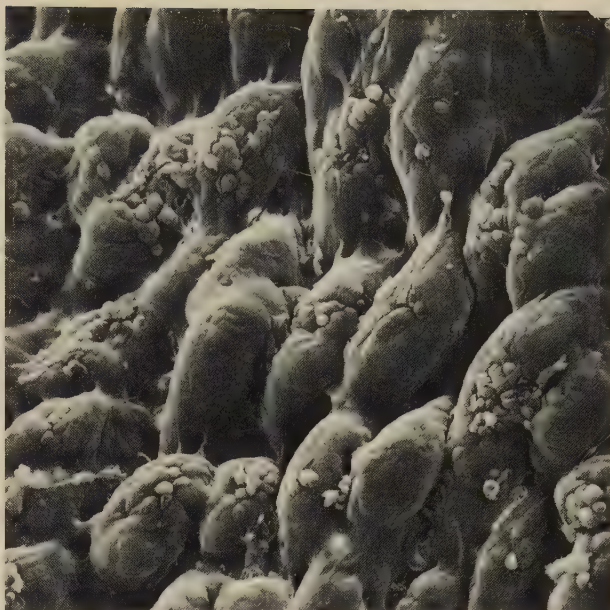


Fig. 53. Score 1.5. 190 ×.



Fig. 54. Score 2.5. 190 ×.

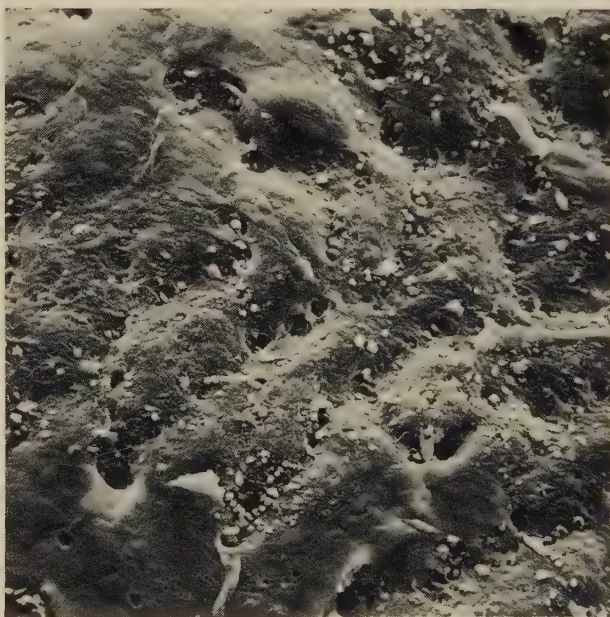


Fig. 55. Score 3.0. 190 ×.

Illustration of the score numbers of the villi.

Epithelial cells.

- 0.0 Normal cells with almost flat surfaces and hexagonal in form; the edges are faintly indicated.
- 0.5 Vaulted surfaces on the functional compartment.
- 1.0 Half-spherical cell surfaces where the edges of the individual cells that have broken away from other cells can clearly be seen.
- 1.5 Vaulted, spherical surfaces on cells found in large clusters in the villus tops. Flat cells are seen on the sides and in the tops of a few villi.
- 2.0 Slightly vaulted separated cells. On the side are seen flat cell surfaces heterogeneous in shape. The cell boundaries are clearly indicated.
- 2.5 Flat cells everywhere with raised, clear cell boundaries. Strong deformation of the cell-shape.
- 3.0 Groups of flat cells with large polygonal cell surfaces having raised cell boundaries, so-called omega cells.
- 3.5 Total denudation.

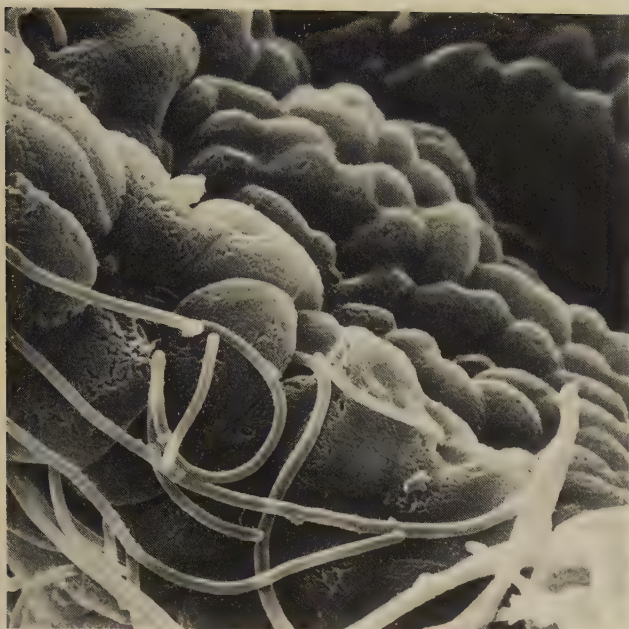


Fig. 56. Score 0.5. 1900 ×.



Fig. 57. Score 1.5. 1900 ×.



Fig. 58. Score 2.5. 1900 ×.



Fig. 59. Score 3.0. 500 ×.

Illustration of the score numbers of the epithelial cells.

Microvilli

- 0.0 Normal, tight, smooth carpet of microvilli tops covering the epithelial cell surface. See Chapter IV.
- 0.5 Beginning separation of the microvilli from each other, especially in the villus-tops.
- 1.0 Long, diverging microvilli with a certain swelling of the tops.
- 1.5 Half-long, sparse microvilli with swollen tops; the microvilli tops are leaning against each other, forming a cone.
- 2.0 Short microvilli, one-third of normal length, sharply separated with plump tops, that are leaning against each other.
- 2.5 Extremely short microvilli isolated from each other with free spaces seen between them.
- 3.0 Faintly indicated plump, isolated microvilli. Some epithelial cells lack microvilli.
- 3.5 No microvilli at all.



Fig. 60. Score 0.5. 5000 $\times$.

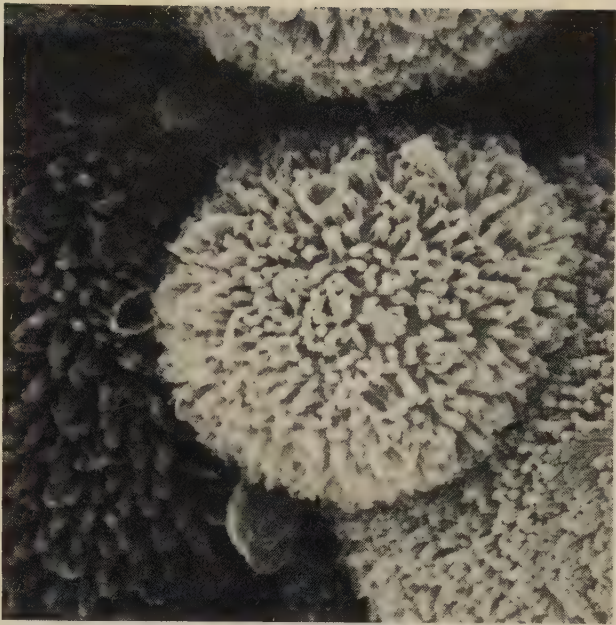


Fig. 61. Score 1.5. 10,000 $\times$.

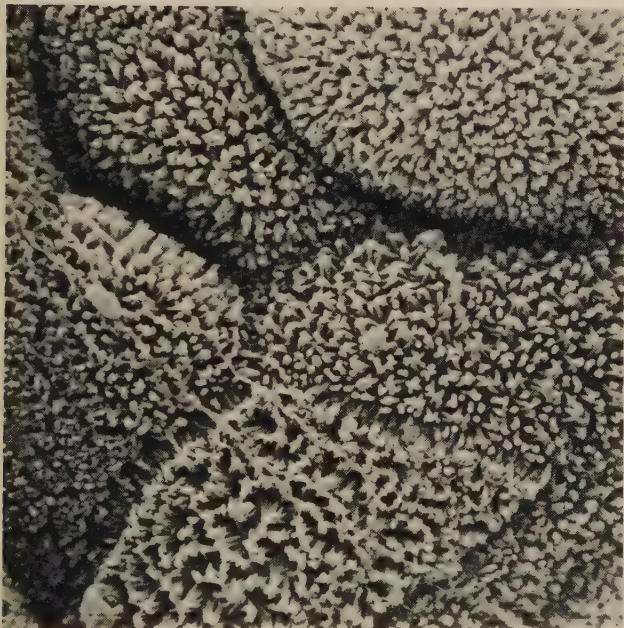


Fig. 62. Score 2.0. 5000 $\times$.



Fig. 63. Score 3.0. 2000 $\times$.

Illustration of the score numbers of the microvilli.

Chapter VII

Single dose irradiation

Introduction

Single doses were given according to the schedule in Table 1 using the experimental model irradiation technique, and the SEM-preparation technique described in Chapter III. LD₍₅₀₎₃₀ in this partial abdominal irradiation study was determined. The immediate reaction and the repair was studied in doses around LD₍₅₀₎₃₀, 22.5 Gy. The single dose schedule was divided into three groups: initial reaction, early reaction and repair.

	Time interval	Dose (Gy)								
		3	6	9	12	15	20	22.5	25	30
Initial reaction	5 min.						x	x	x	x
	10 min.						x	x	x	x
	20 min.						x	x	x	x
	30 min.						x	x	x	x
	60 min.						x	x	x	x
Early reaction	2 hours	x	x	x	x	x	x	x	x	x
	6 hours	x	x	x	x	x	x	x	x	x
	12 hours	x	x	x	x	x	x	x	x	x
	24 hours	x	x	x	x	x	x	x	x	x
	2 days	x	x	x	x	x	x	x	x	x
	3 days	x	x	x	x	x	x	x	x	x
	5 days	x	x	x	x	x	x	x	x	x
Repair	14 days					x	x	x		
	21 days					x	x	x		

Table 1. The single dose irradiation program in the SEM-study.
x = irradiated and examined animals.

At each time-interval, 10 specimens from two rats were examined by SEM and LM and in some cases by TEM. Two rats were considered enough when the same dose was examined at many different time-intervals. Still, the experiments were doubled. The rats had free access to water and pellets and their weight was controlled every day. The condition of the rats was observed but not documented regularly. Irradiation was given in the morning.

The reaction seen by SEM was scored according to an arbitrary scale as presented in Chapter VI for villus, epithelial cell and microvilli. Physiological phenomena were recorded as the degree of attack by bacteria, frequency of Goblet cells and their activity, the extrusion process and the condition of the so called M-cells of the Peyer's patch. Weight curves were analysed with respect to correlation to morphological reactions. Examination of the influence of fasting and refeeding on the weight in relation to irradiation effect and repair was performed. The hypothesis of the effect of mannose on bacteria, as described in Chapter IV, was tested in doses >20 Gy, which give a heavy attack by bacteria.

In the following pages, examples of irradiation effects as seen by SEM are shown from all three groups of the study. Pictures were taken at five or more different magnifications at each dose and time. It is not possible to show more than a few selected examples.

Initial reaction

Initial reaction

Single dose 20 Gy – 10 min

The duration of irradiation to the animal is about 40 min and during this time the villi tend to be swollen

and heavily attacked by bacteria, which can be visualized already 10 minutes after the end of radiation. The swelling of the individual cells created a deeply folded area of the villus, especially at the extrusion top (Fig. 64), where many cells are seen loosening. There is penetration of bacteria in most

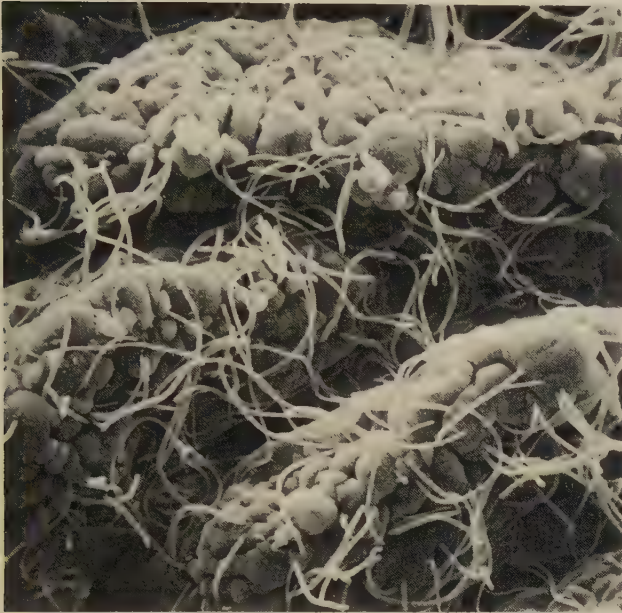


Fig. 64. 20 Gy – 10 min. Single dose – 20 Gy – 10 min. Strong attack of bacteria. Villi are swollen. Cells are leaving the extrusion zone. 190 ×.



Fig. 65. 20 Gy – 10 min. Single dose 20 Gy – 10 min. Top of a villus with penetrating bacteria. 1900 ×.



Fig. 66. 20 Gy – 10 min. Single dose 20 Gy – 10 min. Peyer's patch with a lot of bacteria in the center. 90 ×.

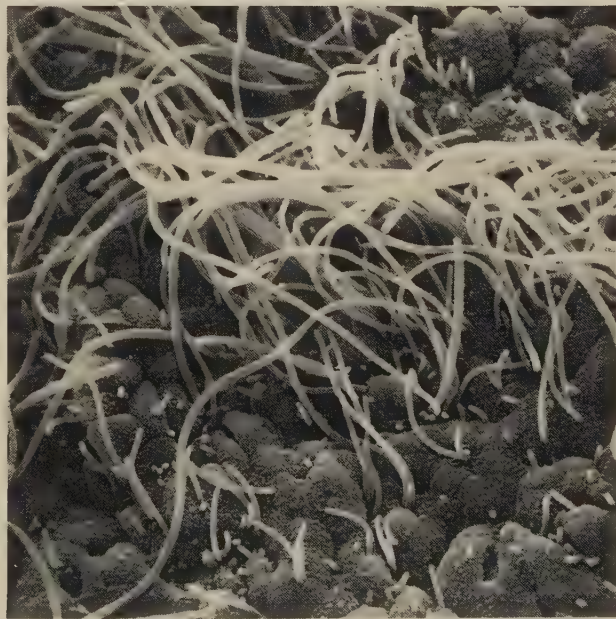


Fig. 67. 20 Gy – 10 min. Single dose 20 Gy – 10 min. The central part of Peyer's patch with bacteria attacking. 900 ×.



Fig. 68. 20 Gy – 10 min. Single dose 20 Gy – 10 min. Intensive attack by bacteria. Mucinous granulae on the surface. 10,000 ×.

epithelial cells at the top and upper third of the villus (Fig. 64). The entrance of the bacteria into the cells seems to take place by chance (Fig. 65). Even at the center of noduli of Peyer's patches, the attack of bacteria is pronounced (Fig. 67). Mucus droplets are spread over the epithelial surface, showing high activity of the Goblet cells.

Eating, non irradiated animals, have bacteria on the Peyer's patches also concentrated to the central area of each dome. The relation between the irradiated animal and the non-irradiated as far as bacteria is concerned is about 3:1 at 10 min after irradiation.

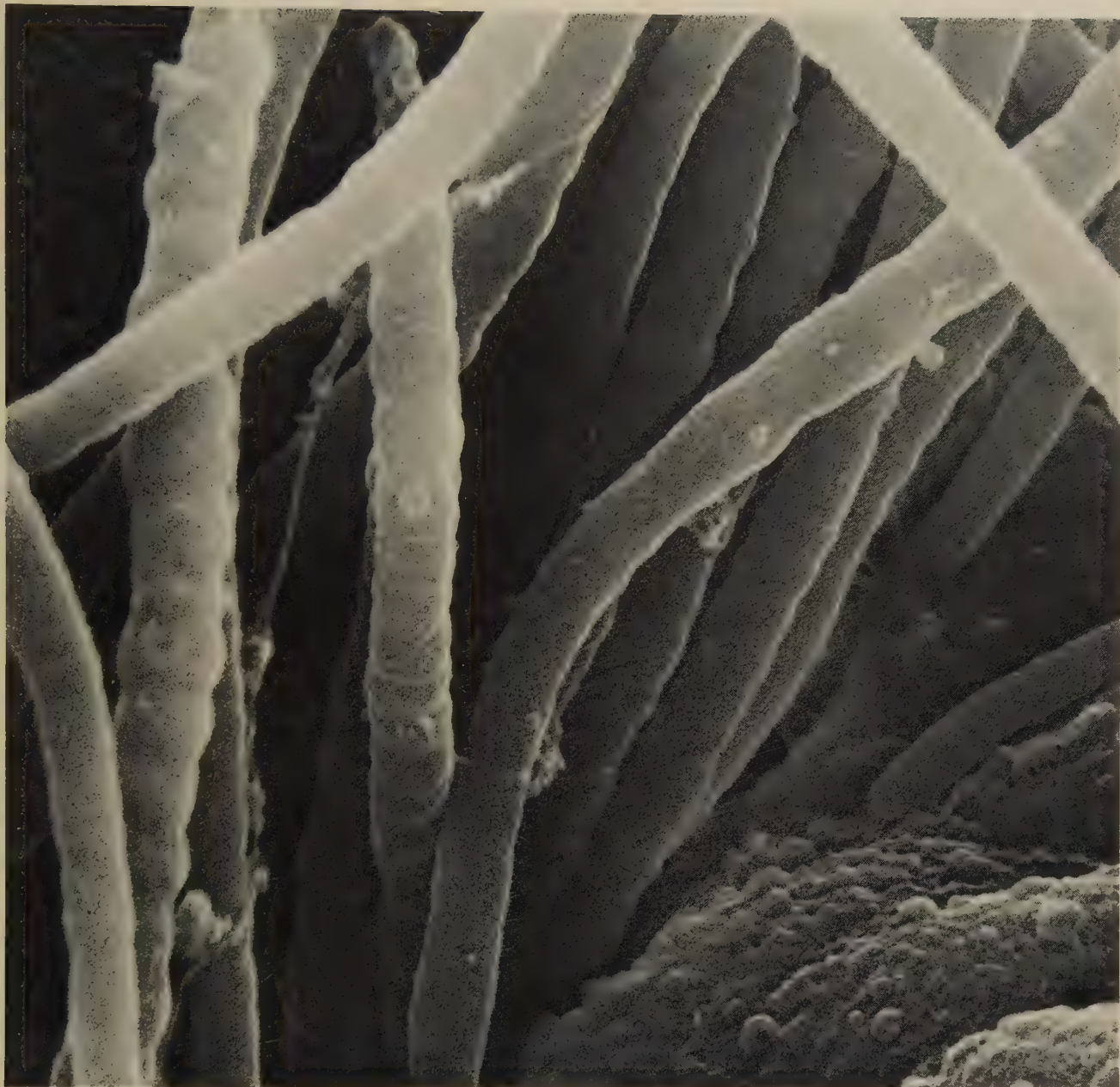


Fig. 69. Single dose 20 Gy – 10 min.
Attack by bacteria at the center of
a nodule of a Peyer's patch in high
magnification. 10,000 $\times$.

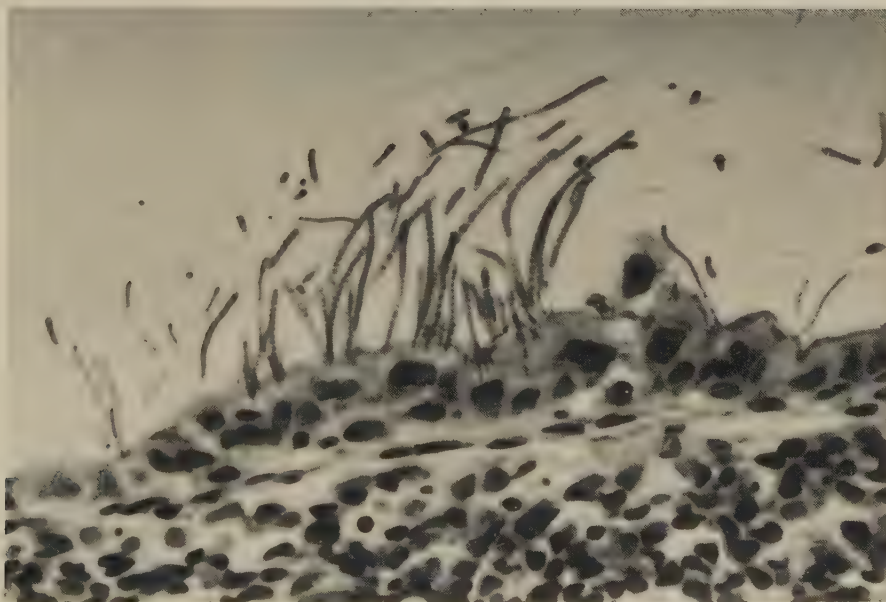


Fig. 70. Single dose 20 Gy – 10 min.
Light microscopy picture from the
center of a nodule of a Peyer's
patch. Large attack by bacteria.

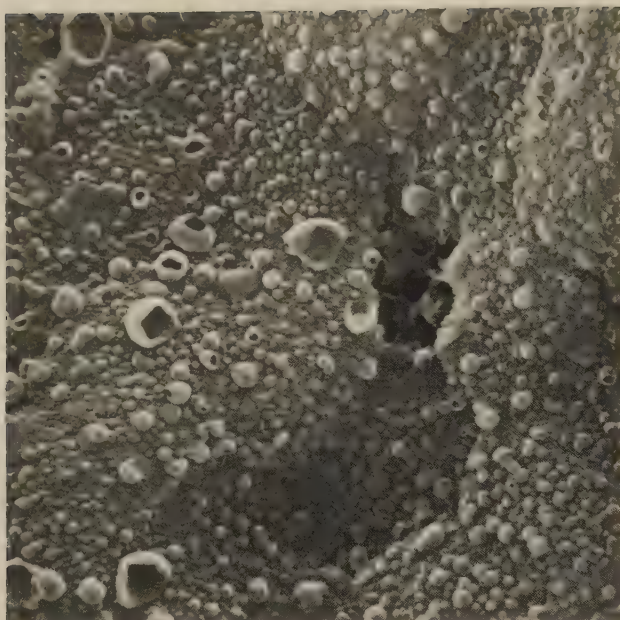


Fig. 71. Single dose 20 Gy – 10 minutes. The cell surface is packed by mucinous granulae of varying size. One Goblet cell opening in the center. 4700 $\times$.

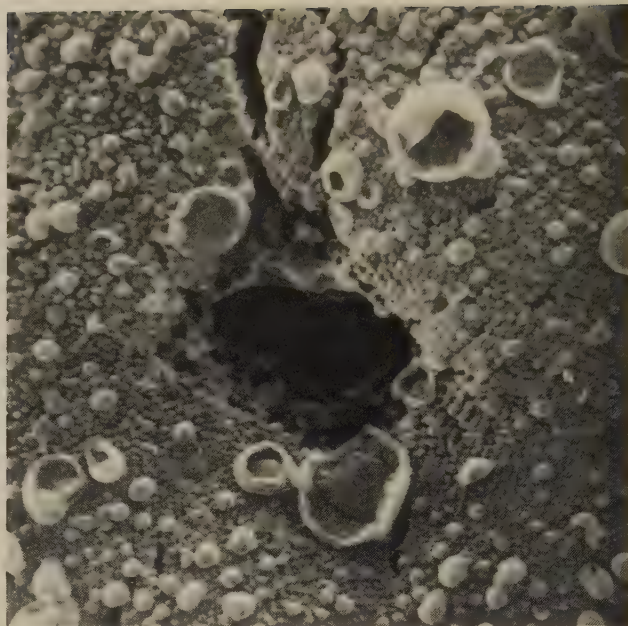


Fig. 72. Single dose 20 Gy – 10 minutes. The Goblet cell is empty. 9000 $\times$.

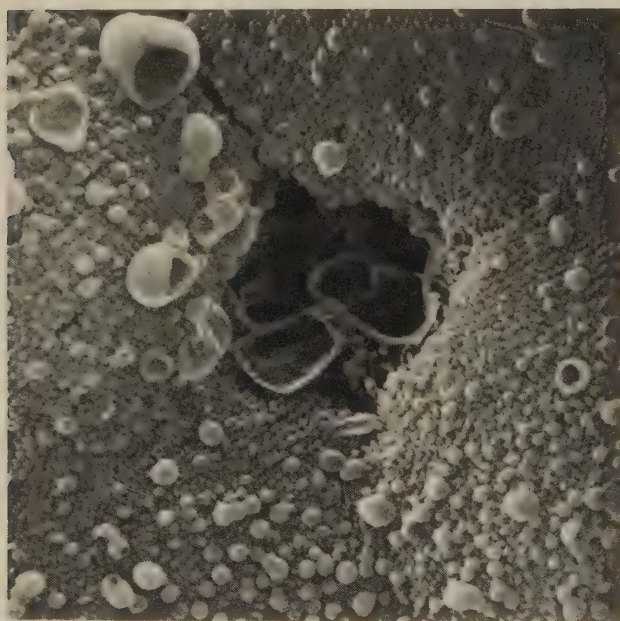


Fig. 73. Single dose 20 Gy – 10 minutes. Shells of granulae still in the cell opening. Tightly packed normal microvilli. 9000 $\times$.

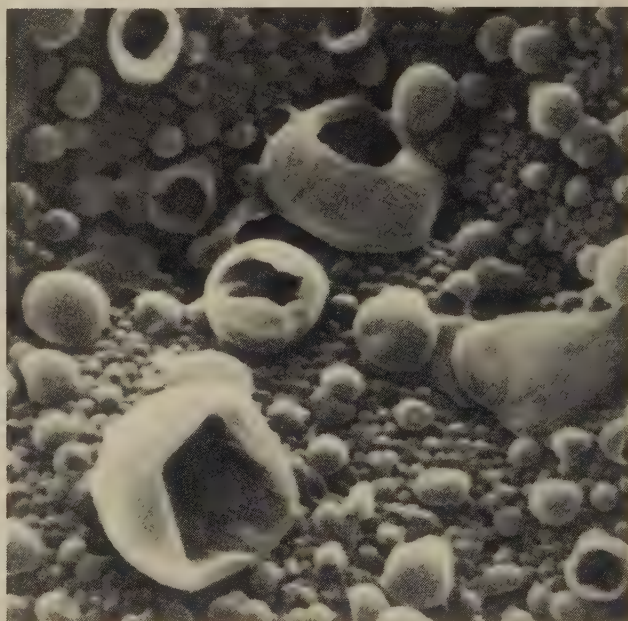


Fig. 74. Single dose 20 Gy – 10 minutes. High magnification of the mucinous granulae with microvilli between them. 19,000 $\times$.

Single dose 20 Gy – 10 minutes

10 minutes after 20 Gy, the Goblet cells explosively throw out their mucinous granulae into the intestinal lumen, as seen in Figs. 71–74. The empty shells vary in size from 1.25 μm to less than 0.2 μm . The framework within the Goblet cells can be seen, and the impression is that the granulae rupture while passing the opening. The epithelial cells have a completely covering microvilli carpet with normal diameter, 0.08 μm . The reaction is normally initiated in non-

fasting animals but is much stronger immediately following high single doses, and also during fractionation, as will be shown after 5 daily fractions of 2 Gy.

All secretion may suddenly cause the Goblet cells to empty, verified by LM-control. It is not known whether the Goblet cells fill up again, or only function once. In fasting animals the Goblet cells migrate up to the extrusion zone and leave the villi unemptied.



Fig. 75. Single dose 25 Gy – 5 min. Villi covered by bacteria. 190 ×.

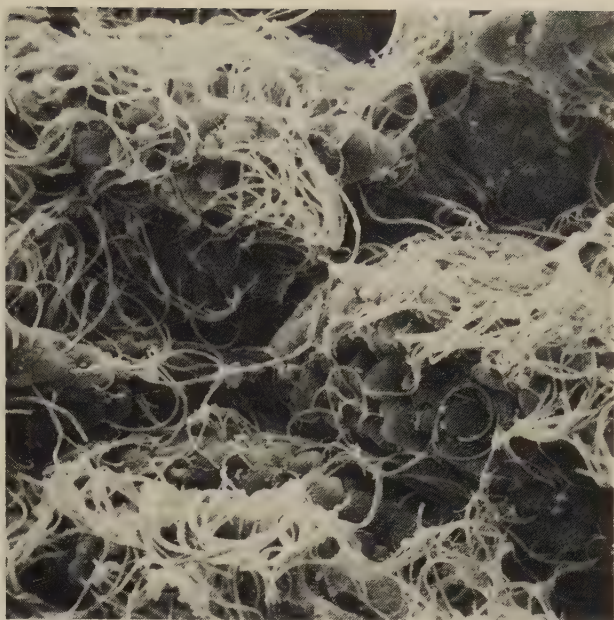


Fig. 76. Single dose 25 Gy – 5 min. Enlargement of Fig. 75. Bacteria on the upper part of the swollen villi. 470 ×.

Single dose 25 Gy – 5 min. – 60 min

Irradiation time was 50 minutes. Five minutes later, there is such a heavy attack by bacteria that it is nearly impossible to identify structures at low magnification. Almost every cell seems to be attacked. (Fig. 75). The bacteria are found on the upper third of the villi, the functional compartment, while the lower part is almost free from attack. The villi

are swollen. This finding of an interaction between the villi and bacteria after irradiation constitutes an important physiological course in relation to the radiation. This consecutive change in the attack of bacteria is followed in a sequence at 5, 10, 30 and 60 minutes after irradiation, and furthermore at 2, 6 and 12 hours. Already 10 minutes after the end of irradiation the bacteria begin to vanish and after 30 minutes they have disappeared completely (Fig. 78).



Fig. 77. Single dose 25 Gy – 5 min. Enlargement of Fig. 75. Many bacteria attacking epithelial cells at the top of a villus. 10,000 $\times$.

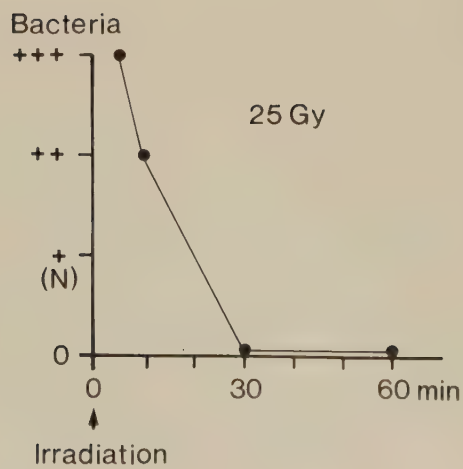


Fig. 78. Frequency of bacteria after completed single dose of 25 Gy scored at different time intervals. +=normal amount of bacteria in the non-fasting rat.



Fig. 79. Single dose 25 Gy – 5 min. One bacterium entering the surface of an epithelial cell. An empty shell of a mucinous granula from a Goblet cell (arrow). 20,000 ×.

The epithelial cells have a convex surface on which the microvilli are irregular, diverging and of different height. Goblet cells are spreading out a lot of mucinous granulae on the cells. The most active cells in the compartment near the villus top may have a high activity as a result of irradiation and an increased blood-flow, resulting in an attractive surface for the bacteria, as suggested in the discussion about bacteria and mannose in Chapter III in non fasting animals. Thus, it may be allowed to

assume the hypothesis that during the long irradiation in this single dose for 50 minutes up to 25 Gy, a kind of stimulation occurs in the mucosa, resulting in a large increase in the bacteria penetration, in oedema seen as swollen cells and in an emptying of the Goblet cells. Soon, or already after 30–60 minutes, the damage has stopped this activity and the following destructive irradiation reaction is started. On the other hand, the cells may be exhausted.

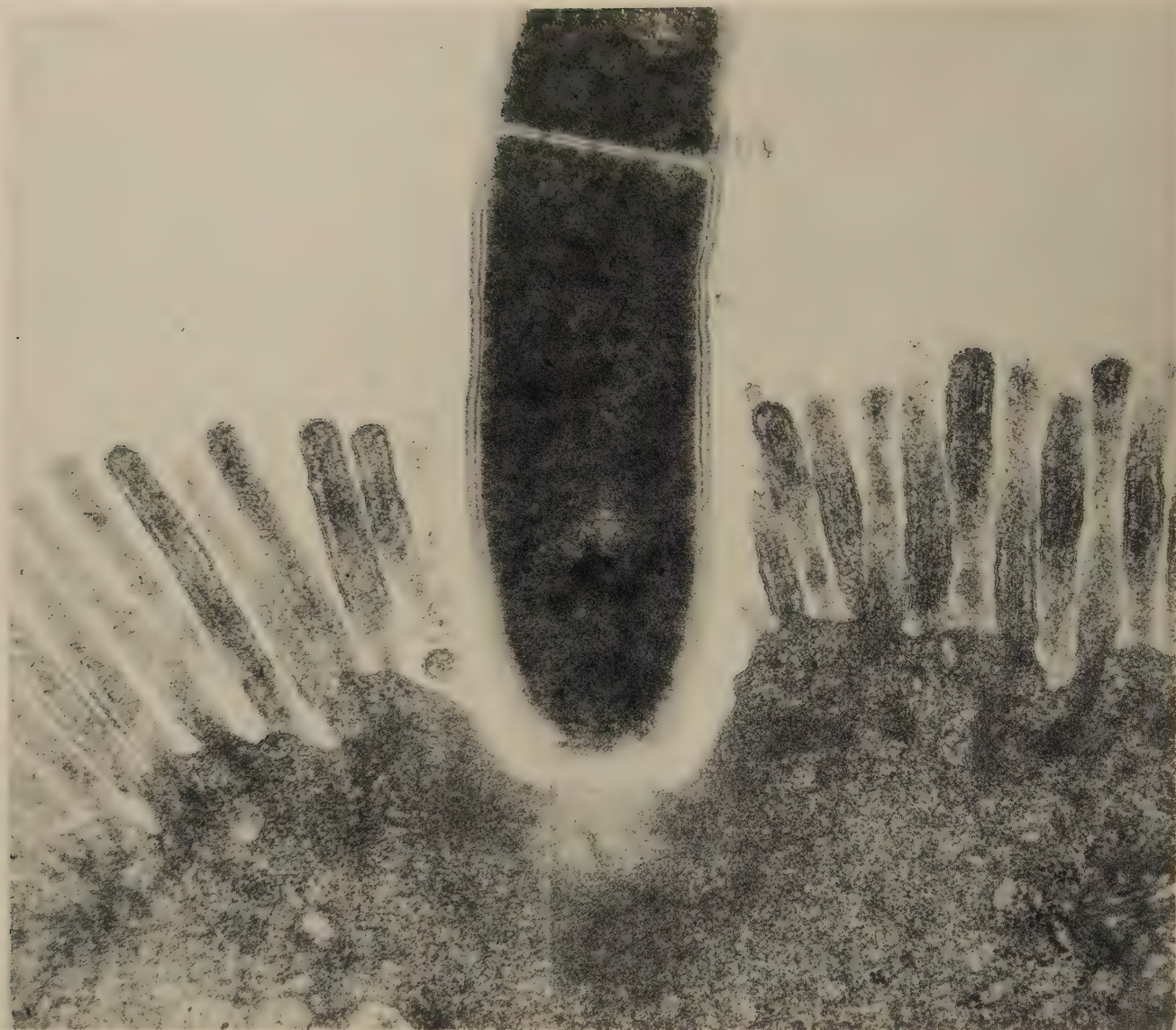


Fig. 80. Single dose 25 Gy – 5 min. TEM-picture of a segmented bacterium penetrating the epithelial cell surface as in Fig. 79. 48,000 $\times$.



Fig. 81, 82, 83. Single dose 25 Gy – 5 min. Bacteria entering epithelial cells of different appearances. The microbes seem to have smaller diameter in the attacking end (arrow). 4700 ×.

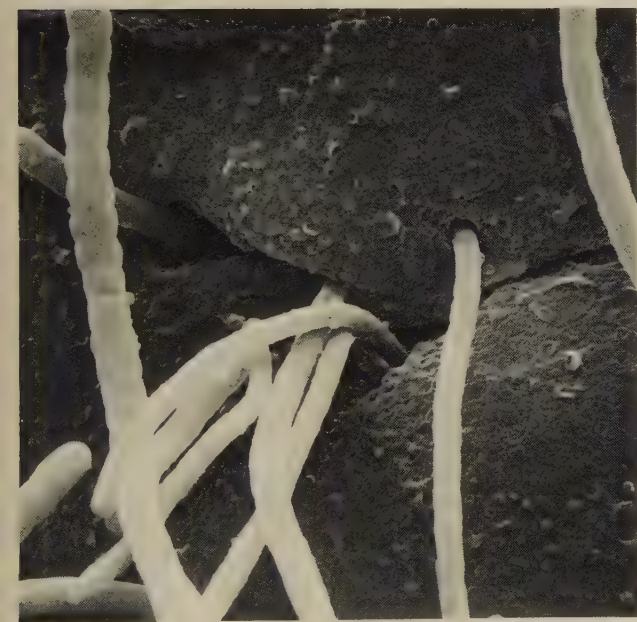


Fig. 84. Single dose 25 Gy – 10 min. In relation to the picture at 5 min. the bacteria have been reduced. Many cells in the extrusion phase (arrow). 470 ×.



Fig. 85. Single dose 25 Gy – 30 min. The villi are still swollen with many folds and a few cells in the extrusion zone. Goblet cell openings are seen as well as mucinous granulae but no bacteria. 470 ×.

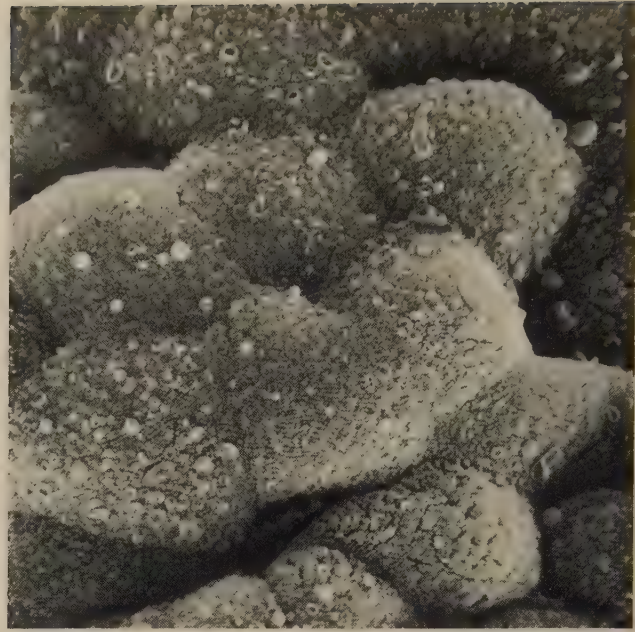


Fig. 86. Single dose 25 Gy – 30 min. Enlargement of Fig. 85. Spherical epithelial cells with regular rows of microvilli and a lot of mucinous granulae over the cells. 4700 ×.



Fig. 87. Single dose 25 Gy – 30 min. Peyer's patch. No bacteria are seen. No cells in extrusion. A rich amount of M-cells are seen on the surface. 190 ×.



Fig. 88. Single dose 25 Gy – 60 min. Still no bacteria. The morphologic appearance about the same as 30 minutes after irradiation. 900 ×.

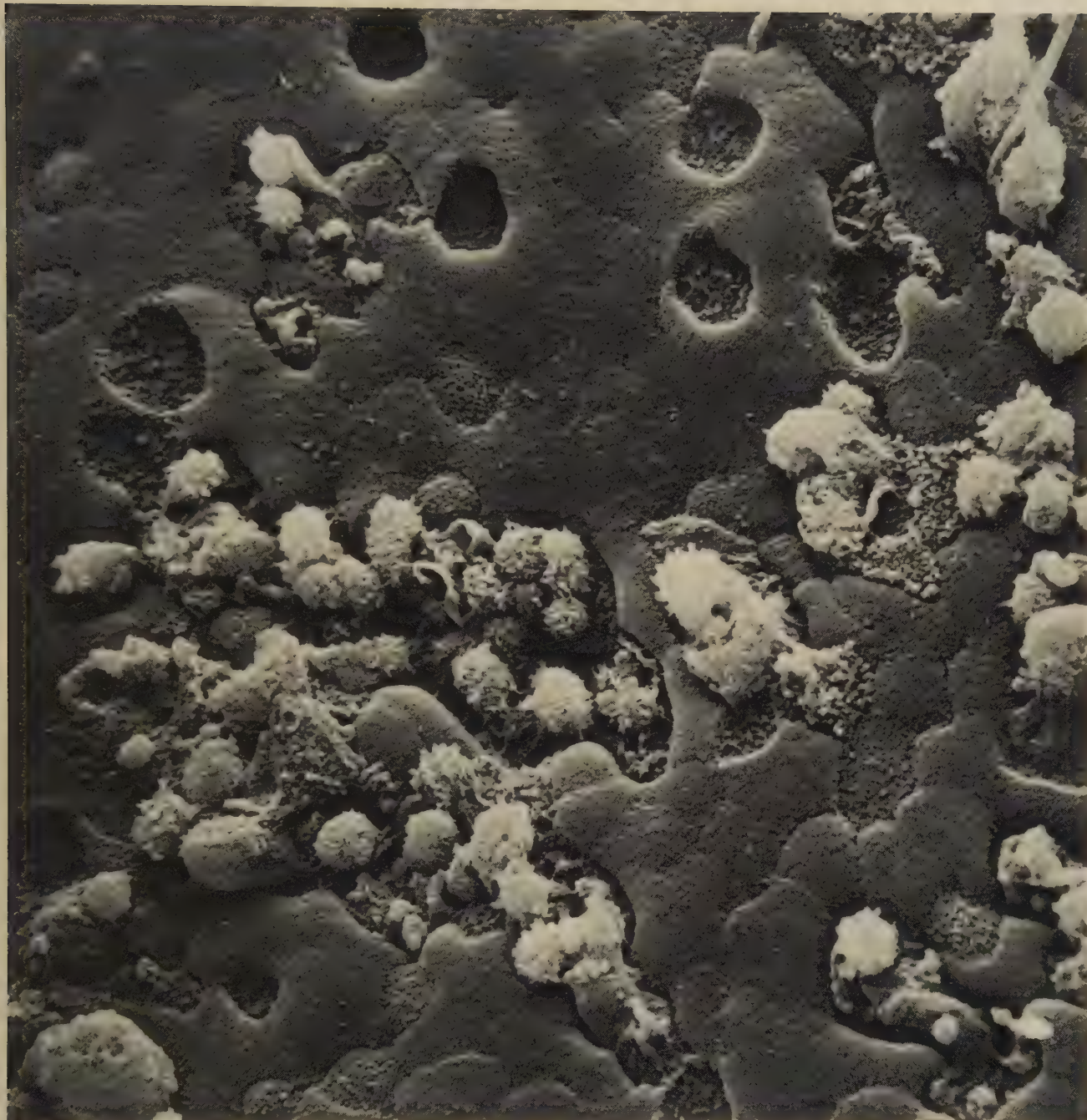


Fig. 89. Single dose 30 Gy – 5 min. Peripheral area of a Peyer's patch. Many of the M-cells have penetrating lymphoid cells. 1900 ×.

Single dose 30 Gy – 5 min

A partial irradiation of the abdomen with 30 Gy is a lethal dose for the rats. In addition to the effect on the morphology of such high doses as 25 Gy, other things have been observed at the Peyer's patches, especially in the peripheral area.

The time for giving 30 Gy is about 60 min, and 5

min after the irradiation many M-cells in the Peyer's patches show lymphoid cells in their openings. Some of these cells have already penetrated the M-cell membrane; others are starting to do it. Fig. 89 illustrates this phenomenon. Most of the cells which have penetrated have short scattered protrusions.



Fig. 90. Single dose 30 Gy – 5 min. Closed M-cell with a few microvilli-like structures on the surface. 9000 $\times$.

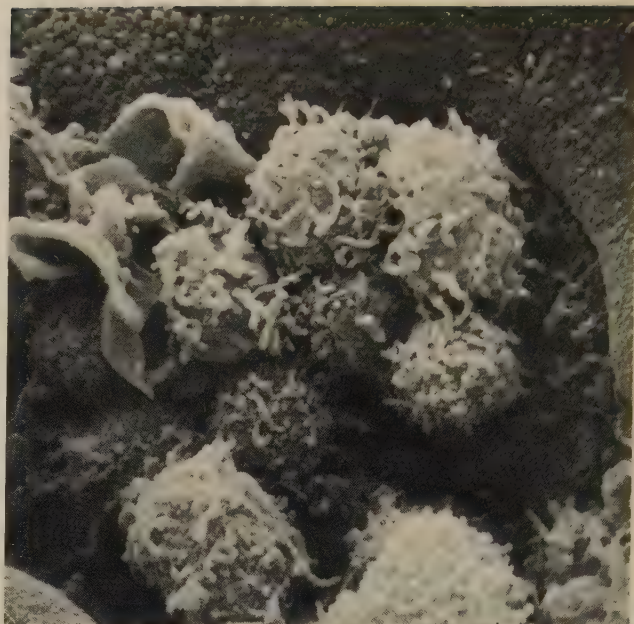


Fig. 91. Single dose 30 Gy – 5 min. Lymphoid cells penetrating. Note the number of cells and the holes behind. 4700 $\times$.

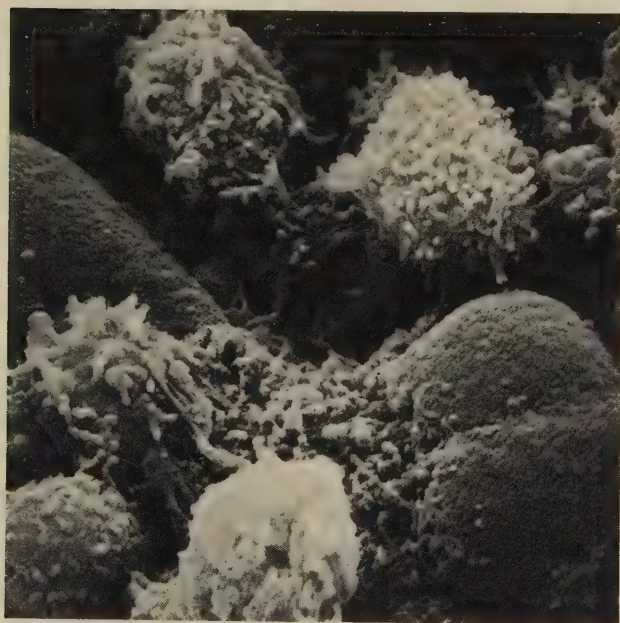


Fig. 92. Single dose 30 Gy – 5 min. Another close-up of the reaction. 4700 $\times$.

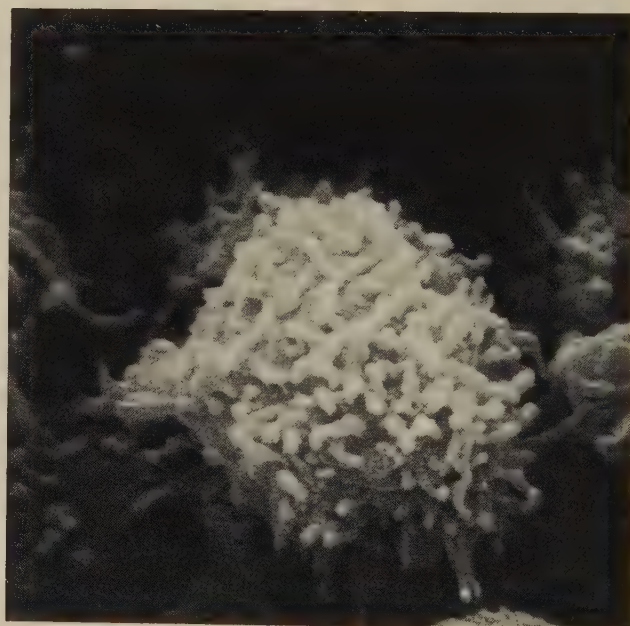


Fig. 93. Single dose 30 Gy – 5 min. Lymphoid cell, with protrusions. Diameter about 5 μm . 9000 $\times$.

Viewing the area of the Peyer's patches the lymphoid cells are in different "stages". Some M-cells are closed (Fig. 90), showing a surface with short microvilli-like structures. Others have been opened and lymphoid cells are pouring out into the lumen. Empty holes through which the cells have passed

are seen in Fig. 91. The diameter of the free cells is about 5 μm and the diameter of the holes is smaller.

As seen in Chapter IV in fasting animals the whole dome surface of the Peyer's patch is clean and empty but in non-fasting animals a few cells are seen coming up from the cell surface.

Early reaction

Single dose 2 Gy – 48 hours

Villi are of normal height and form, but some tend to have triangular tops. There is massive attack by bacteria over the extrusion zones and the upper parts. Extrusion activity cannot be seen because of the bacteria. Folds are deep, which is a sign of oedema of the epithelial cells. (Fig. 94). In high magnification the multiple bacteria penetrating the cells are seen together with empty mucinous droplets on the cell-surface, where the microvilli are normal,

long and with no swelling of the tips. (Fig. 95). This low single dose is regarded as a sign of stimulation (cf. 20 Gy – 10 min). The Peyer's patches show the same situation, previously demonstrated in "initial" reaction after 30 Gy, with heavy attack by bacteria in the center and with penetration of the surface by lymphoid cells. At this low single dose, Figs. 96 and 97 demonstrate the same thing. The narrow holes in the M-cells have a diameter of about 1.5 μm , too small for the lymphoid cells. They will have to change form while slipping through. The steps have been shown in connection with single dose 30 Gy after 5 minutes.



Fig. 94. Single dose 2 Gy – 48 hours. Swollen villi heavily attacked by bacteria. 430 $\times$.



Fig. 95. Single dose 2 Gy – 48 hours. Many bacteria attacking at the same point. Normal microvilli. Mucinous granulae on the surface. 9000 ×.

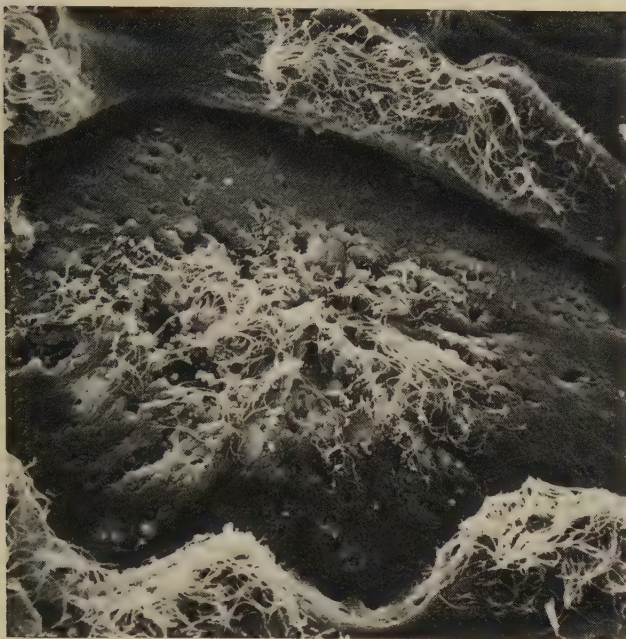


Fig. 96. Single dose 2 Gy - 48 hours, Peyer's patch. A lot of bacteria in the central area and on the surrounding villi. The surface is broken up here and there with free cells visible. 200 ×.

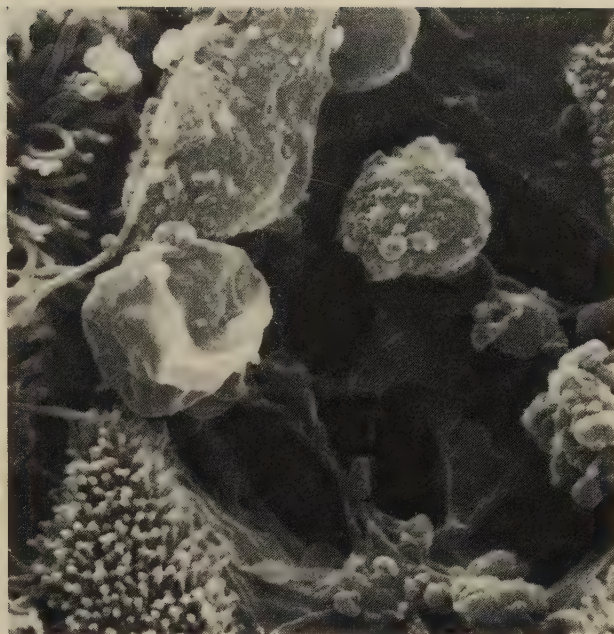


Fig. 97. Single dose 2 Gy - 48 hours. M-cell with no membrane but with empty holes. See 30 Gy - 5 min. 10,000 ×.

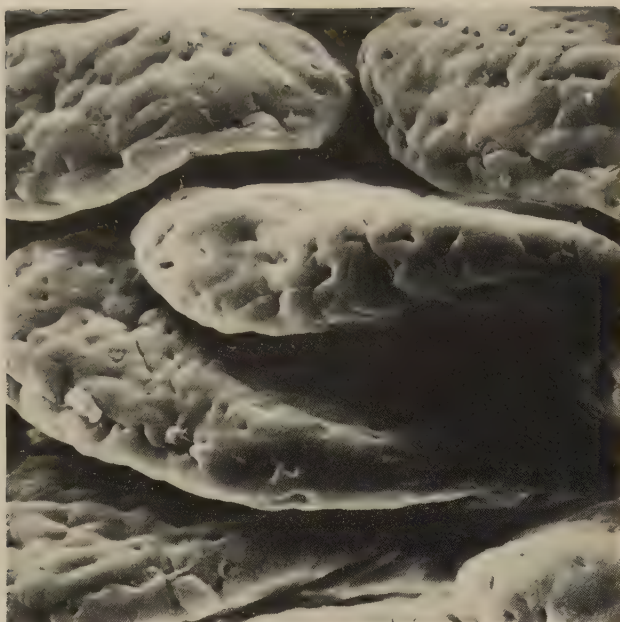


Fig. 98. Single dose 3 Gy – 6 hours. Swollen villi. Goblet cells are seen up to the top of the villi. No bacteria. 470 ×.



Fig. 99. Single dose 3 Gy – 6 hours. Side of a villus. Swollen cells. Goblet cells in different extrusion phases. 1900 ×.

Single dose 3 Gy – 6 hours

The villi are swollen with a few loosening cells in the extrusion zones. The epithelial cells are bulky on the surface. Goblet cell openings can be seen right up to

the extrusion zone. The microvilli are slightly irregular with varying diameter. No bacteria are seen.

Single dose 6 Gy – 48 hours – 3 days – 5 days – 7 days

A heavy attack by bacteria is seen after 48 hours on otherwise rather normal villi. (Fig. 100). 24 hours later, at 3 days after irradiation, the villi are swollen, pyramidal in form and only a few villi have bacteria on the tops. (Fig. 101). At 5 days, the picture is more like the situation after 48 hours. The

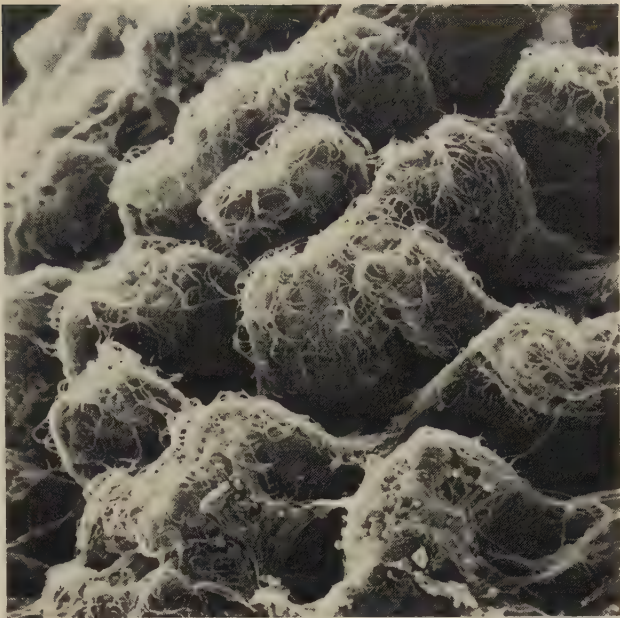


Fig. 100. Single dose 6 Gy – 48 hours. Villi under a heavy attack by bacteria. 200 ×.

Fig. 101. Single dose 6 Gy – 3 days. Swollen villi. Pyramidal form. A few bacteria on some villi. 400 ×.



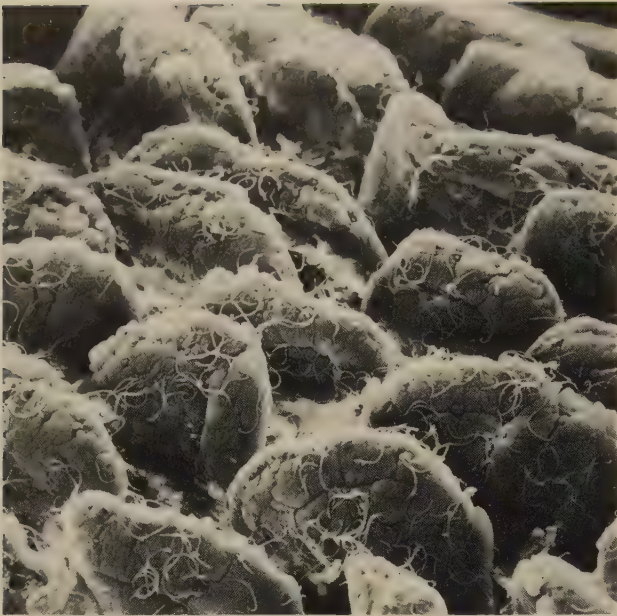


Fig. 102. Single dose 6 Gy – 5 days. Increased attack by bacteria. Swollen villi. 190 ×.



Fig. 103. Single dose 6 Gy – 7 days. Normalized villi but heavy attack by bacteria. 190 ×.

villi may still have a triangular form but the bacteria are again present (Fig. 102). 7 days after 6 Gy there is an intensive bacterial activity on the tops of the villi. Most cells in this region are spherical in form with divergent microvilli. Figs. 103–104).

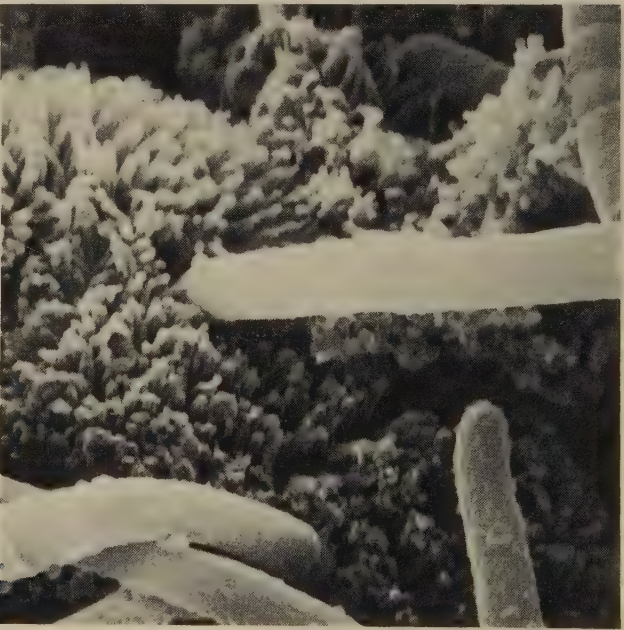


Fig. 104. Single dose. 6 Gy – 7 days. High magnification of Fig. 103. Bacteria and sparse microvilli. 9000 ×.

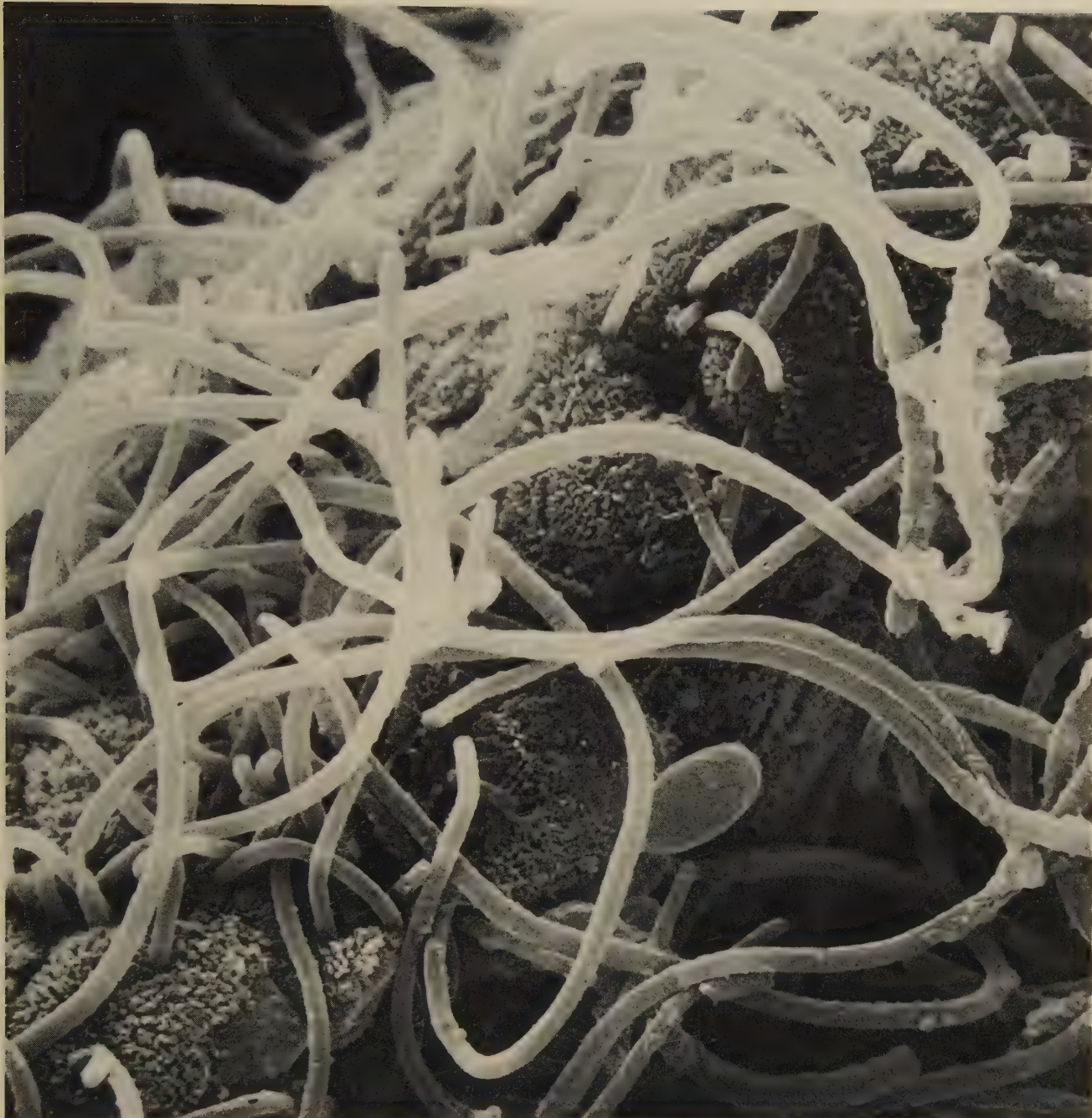


Fig. 105. Single dose 6 Gy – 7 days. Intensive bacterial activity. Swollen cells. 4000 ×.



Fig. 106. Single dose 9 Gy – 6 hours. Thin villi without a real extrusion zone. Goblet cell openings are seen even at the top. No activity in the mucus production can be seen and there is no attack of bacteria. 190 $\times$.

Single dose 9 Gy – 6 hours

6 hours after 9 Gy as a single dose the villi were slender and leaf-like and had an appearance like that in the fasting animal. Single villi, however, had achieved a tendency to triangular form. The height of the villi was within the normal limits. No cells in the extrusion phase were seen (Fig. 106).

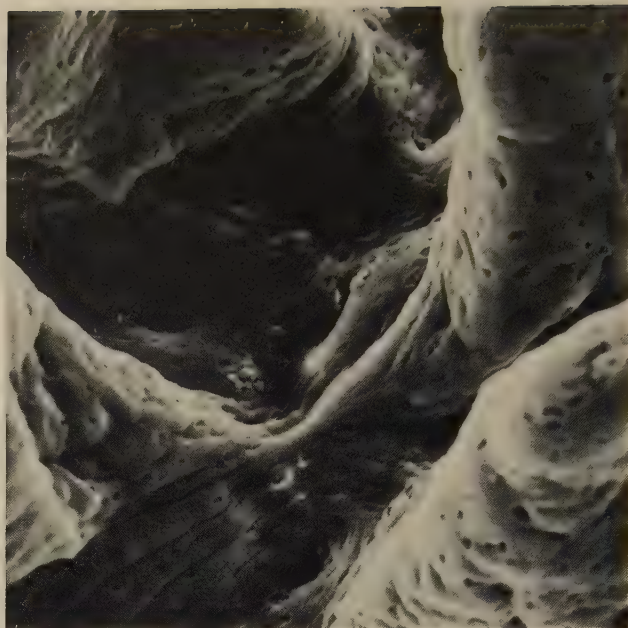


Fig. 107. Single dose 9 Gy – 6 hours. Between the villi the bottom is clearly seen with ridges from the one villus to the other. Goblet cells without mucus extrusion. 470 $\times$.

Folds were not clearly visible. Goblet cell openings were scattered over the whole surface without signs of being emptied. These openings could be followed all the way to the top of the villus, a state which is consistent with the picture of a fasting animal. The bottom between the villi could be observed since there was no oedema (Fig. 107). No bacteria.



Fig. 108. Single dose 9 Gy – 3 days. Swollen, packed villi, triangular in form. Increased extrusion on the tops (arrow). No Goblet cells or bacteria. 1000 ×.

Single dose – 9 Gy – 3 days

The maximum effect is seen on SEM on the third day. The villi are closely packed together, swollen and with shallow folds. The height is shorter than normal. Fig. 108. The form is triangular at the top

with clusters of loosening epithelial cells at only one location. These cells are spherical, separated from each other, swollen. No Goblet cells, emptied or not emptied, are seen, and no bacteria at all.

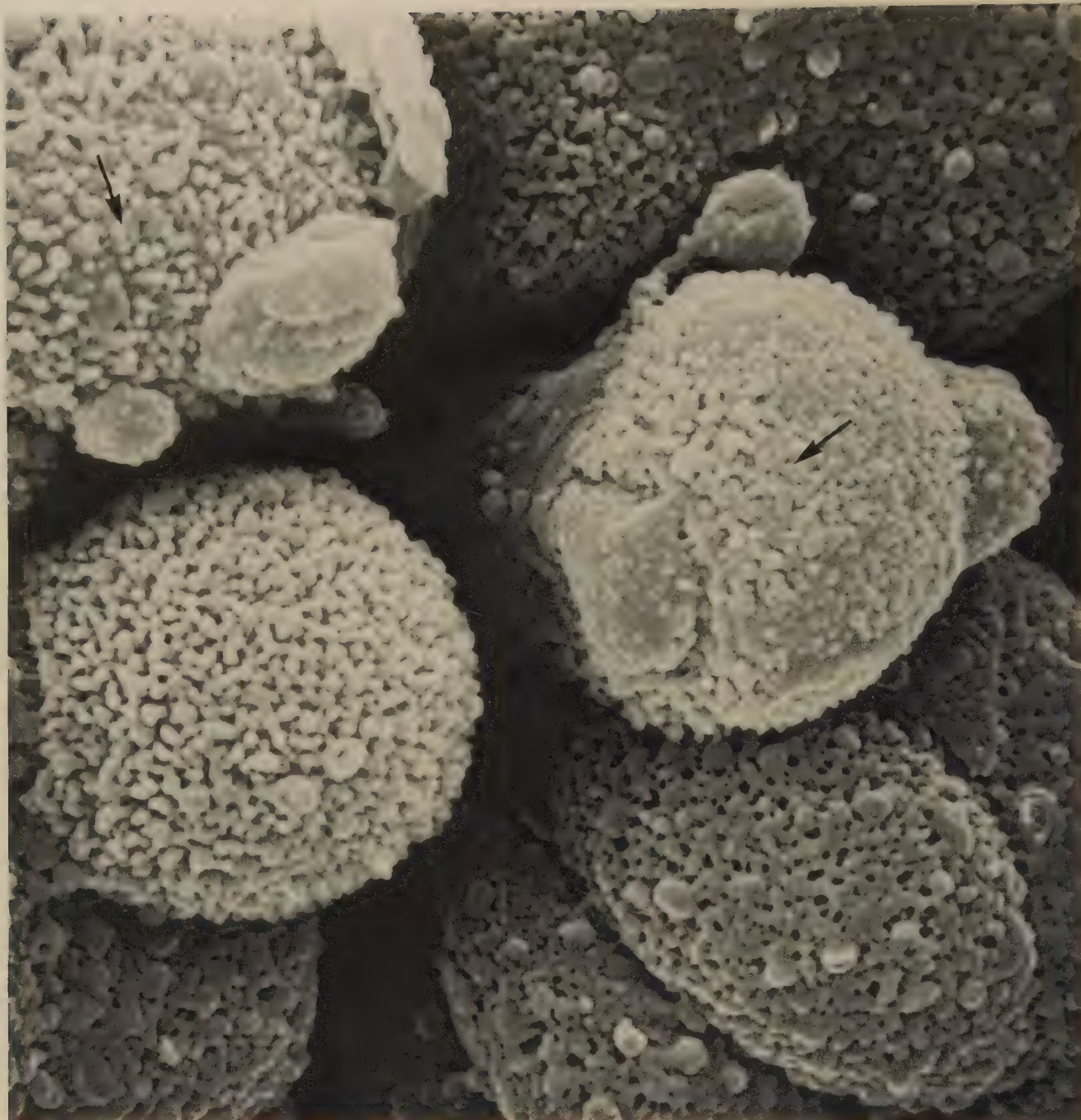


Fig. 109. Single dose 9 Gy – 3 days. Extrusion zone. Cells divided from each other with spherical surface. The microvilli are sparse (arrow). The diameter of the microvilli is about twice that of normal: $0.16\ \mu\text{m}$. $10,000\times$.

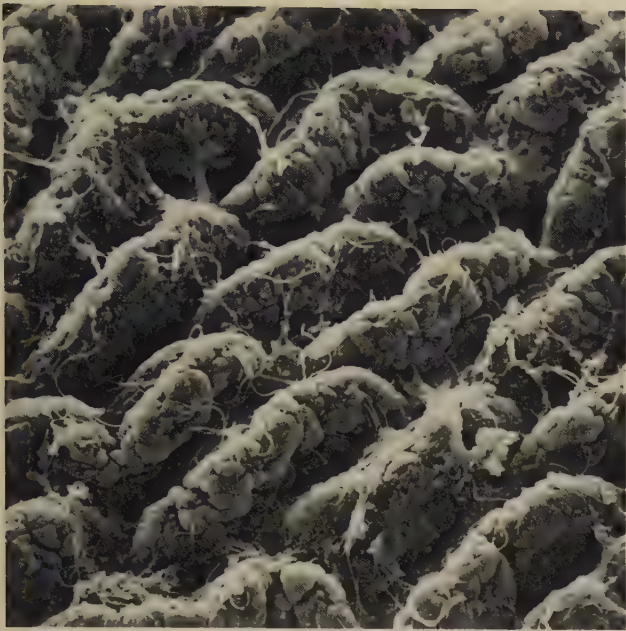


Fig. 110. Single dose 9 Gy – 5 days. Retracted, swollen villi, with normal top. Bacteria are back again. 200 ×.



Fig. 111. Single dose 9 Gy – 5 days. Bacteria penetrating epithelial cells. The microvilli covering is uneven. 10,000 ×.



Fig. 112. Single dose 9 Gy – 7 days. Normal surface of Peyer's patch. Mucinous granulae (arrow). A kind of bacteria located only on the M-cells (two arrows). 4700 ×.

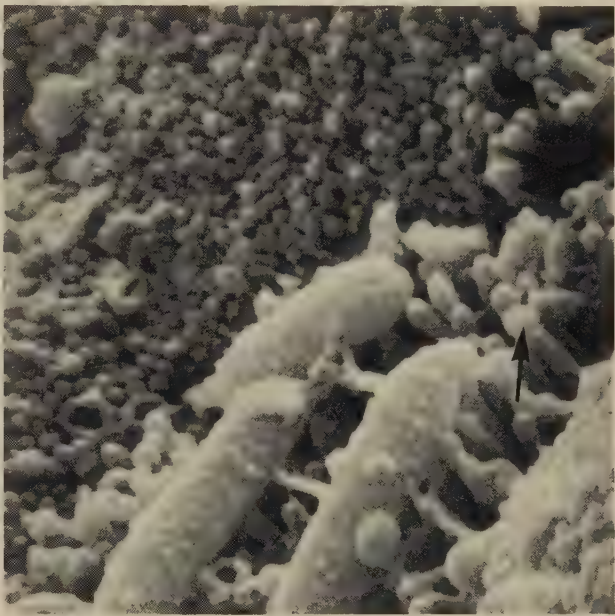


Fig. 113. Single dose 9 Gy – 7 days. Part of Fig. 112, showing the bacteria and the long, sparse, in the end club-like microvilli on the M-cell. 19,000 ×.

Single dose – 9 Gy – 5 days

Already on the fifth day the villi have regained their normal height and form. There are no more triangular tops, but long ridges. The epithelial cells are still separated from each other on the upper third part and more swollen than before. Otherwise the picture is not far from a retracted villus in a non fasting animal. The amount of bacteria is back to normal. The tips of the microvilli do not form a tight carpet,

but the diameter is normal. In Fig. 112 the Peyer's patch has a normal surface with mucinous droplets and some bacteria concentrated only to the membranes of the so called M-cells. About the dose 9 Gy, see also Chapter VIII in the fractionation schedule of 1 F/week = 9 Gy/week.

Single dose – 9 Gy – 7 days

Normal villi, cells and microvilli.



Fig. 114. Single dose 12 Gy – 3 days. Villi are short, long and slender with extrusion-zones (arrow) or plain intact convex tops. The individual epithelial cells are big and polygonal (a). No folds, goblet cells or bacteria are seen. 400 ×.

Single dose 12 Gy – 3 days

The reaction at 3 days after 12 Gy is quite different from that at 3 days after 9 Gy. The maximum effect seen by SEM on the mucosa is reached at the same time as after 9 Gy, but now the villi are much shorter. This, as seen in Fig. 114, results in long, slender villi, because the base of the normal villus is longer than the top. Most of them have small groups of cells in extrusion (arrow) but some villi are intact

on their convex plain surface. There are no longer any cells that can loosen. Folds are not seen and neither are the Goblet cells or the bacteria. The epithelial cells are polygonal on the intact surface with a big, plain surface. The low villi are swollen and the bottom between them is not visible. About the same reaction in score number on the arbitrary scale is seen at 3 days after 15 Gy, and 20 Gy. LM-



Fig. 115. Single dose 12 Gy – 3 days. High magnification of cells separated in the extrusion-zone from one villus. The spherical surface is obvious with short, spread microvilli with club-formed tips. These cells are just penetrating into the intestinal lumen. 10,000 ×.

control shows cuboidal or squamous, flat cells covering the surface. The increased reaction from 9 to 12 Gy and higher doses demonstrates a step to a situation where the villus must react through a dramatic shrinking of the villus by increased massive extrusion of cells at the top (until no more cells can loosen any more and the top is closed – being flat as in Fig. 114).

The epithelial cells lying in groups on some of the villi being extruded are shown in Fig. 115. Those cells differ from the remaining covering cells and are spherical with microvilli spread, showing the bottom between them. The microvilli on the cells holding the surface intact have shorter microvilli. The microvilli in Fig. 115 have a diameter twice as large as normal = $0.16\ \mu\text{m}$.



Fig. 116. Single dose 15 Gy – 3 days. Short villi with smooth surface, some with extended extrusion zone. Crypts of Lieberkühn are seen. 400 ×.

Single dose 15 Gy – 3 days

Villi are characterized by bulky, humpformed ridges. They are shorter in length. Some villi have an extrusion zone with clusters of loosening cells. On the other hand, some have an intact, flat surface on the top (Fig. 116). On the sides, folds are lacking. The strictly symmetrical arrangement of the villi as normally seen is not so sharply defined. The number per square unit, however, can still be counted and is normal. Bridges between the villi are seen. With higher magnification the epithelial cells are seen to have a spherical form in the extrusion zone (Fig. 117) and are conglomerated on the top of the villi. The single cells are separated from each other and are

covered by a surface with microvilli, which in comparison with normal microvilli are shorter and adhere together at the tips. Even on the side of the villi, the epithelial cells have shorter microvilli (about 50% of the normal length as checked by TEM). The density is also less than on the normal cell. Instead of 100 microvilli per μm^2 a value down to 50 per μm^2 will be found. (Fig. 117.)

Bacteria cannot be detected and Goblet cell openings are lacking. The crypts of Lieberkühn, however, can be demonstrated (Fig. 116).

For comparison a LM picture was taken (Fig. 118) where the cells in extrusion are clearly seen at the top of the villi.



Fig. 117. Single dose 15 Gy - 3 days. Enlargement of Fig. 116. Spherical cells with short microvilli stuck together at their tips. 10,000 $\times$.

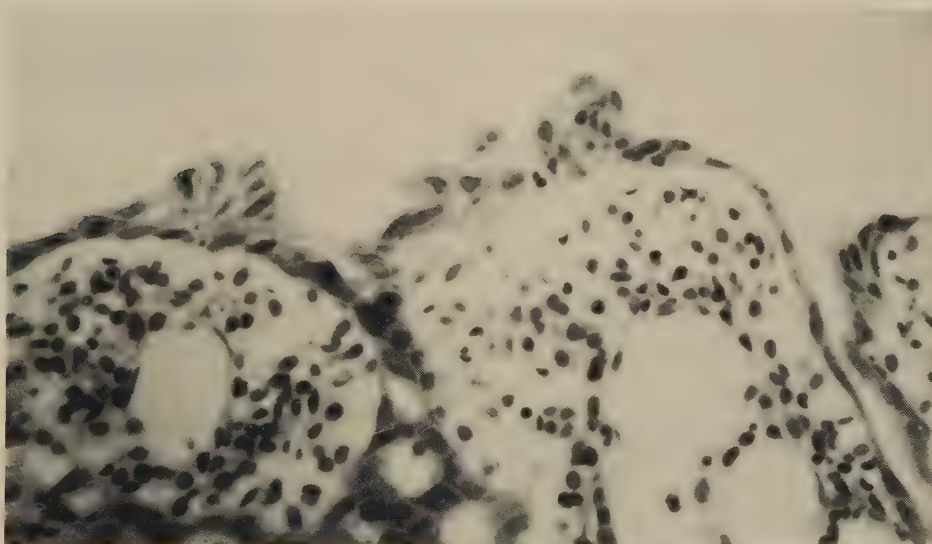


Fig. 118. Single dose 15 Gy - 3 days. Light microscopy picture comparable to Fig. 116.



Fig. 119. Single dose 15 Gy – 5 days. Irregular pattern of the villi. Clusters of loosening cells. No bacteria or Globet cells or mucus droplets. 200 ×.

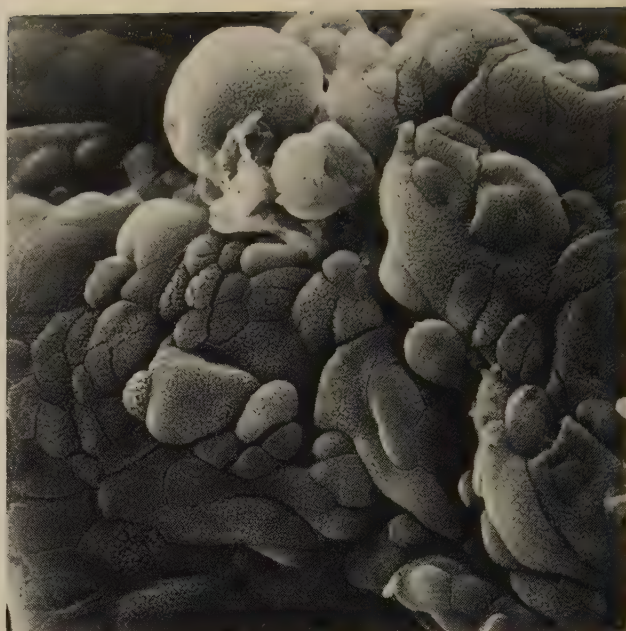


Fig. 120. Single dose 15 Gy – 5 days. Part of Fig. 119 showing distinct borderlines between the cells. 1000 ×.

Single dose 15 Gy – 5 days

Five days after a single dose of 15 Gy the villi have changed a little in comparison with the situation on the third day. As a whole, the villi are more irregular (Fig. 119). On some other places the picture is like Fig. 120. Extrusion of cells is seen to about the same extent as after 3 days. At higher magnification the epithelial cells are spherical and at the top of the villus to a certain degree isolated from each other (Fig. 121). The explanation for this physiological situation may be that it is a mixture of disintegration and at the same time a repair process. The pictures are very difficult to differentiate from those taken 3 days after radiation. Goblet cells cannot be seen, nor mucus granulae from such cells. Bacteria are not present.

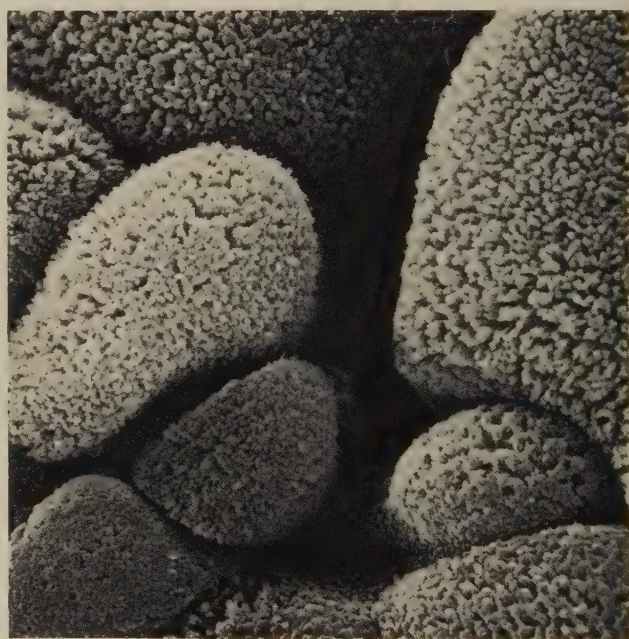


Fig. 121. Single dose 15 Gy – 5 days. Part of Fig. 3. Separated microvilli on spherical cell surfaces, partly isolated from each other. 5000 ×.



Fig. 122. Single dose 15 Gy – 7 days. Villi are short with bullous cells. 190 ×.



Fig. 123. Single dose 15 Gy – 7 days. Swollen epithelial cells on the side of a villus. 900 ×.



Fig. 124. Single dose 15 Gy – 7 days. Microvilli with adhesive tops. 5000 ×.

Single dose 15 Gy – 7 days

Very little change in the structural appearance in comparison to the fifth day (Fig. 122). The villi are of the same low type covered by spherical epithelial cells, making the side wall ball-structured. Extruding cells are seen. At higher magnification the pictures are the same as after 5 days at 15 Gy. The same can be seen regarding the microvilli. The average score-number for the effects after 3, 5 and 7 days has therefore been evaluated to 1.5.



Fig. 125. Single dose 20 Gy – 3 days. Villi are short, mostly flat, with different forms of cells (arrow). Extrusion is still going on, on some of the villi. No folds, goblet cells or bacteria. 400 ×.

Single dose 20 Gy – 3 days

Three days after 20 Gy as a single dose the mucosa has changed to very low and swollen villi, where the extrusion process is going on here and there. The villi are covered by big polygonal epithelial cells

over the slender, flat surface. Loosened free cells are spread everywhere. The SEM picture resembles that of three days after 12 and 15 Gy. Thus, folds are lacking as well as goblet cells and bacteria. The cells, Fig. 125 (arrow), are of the squamous cell type

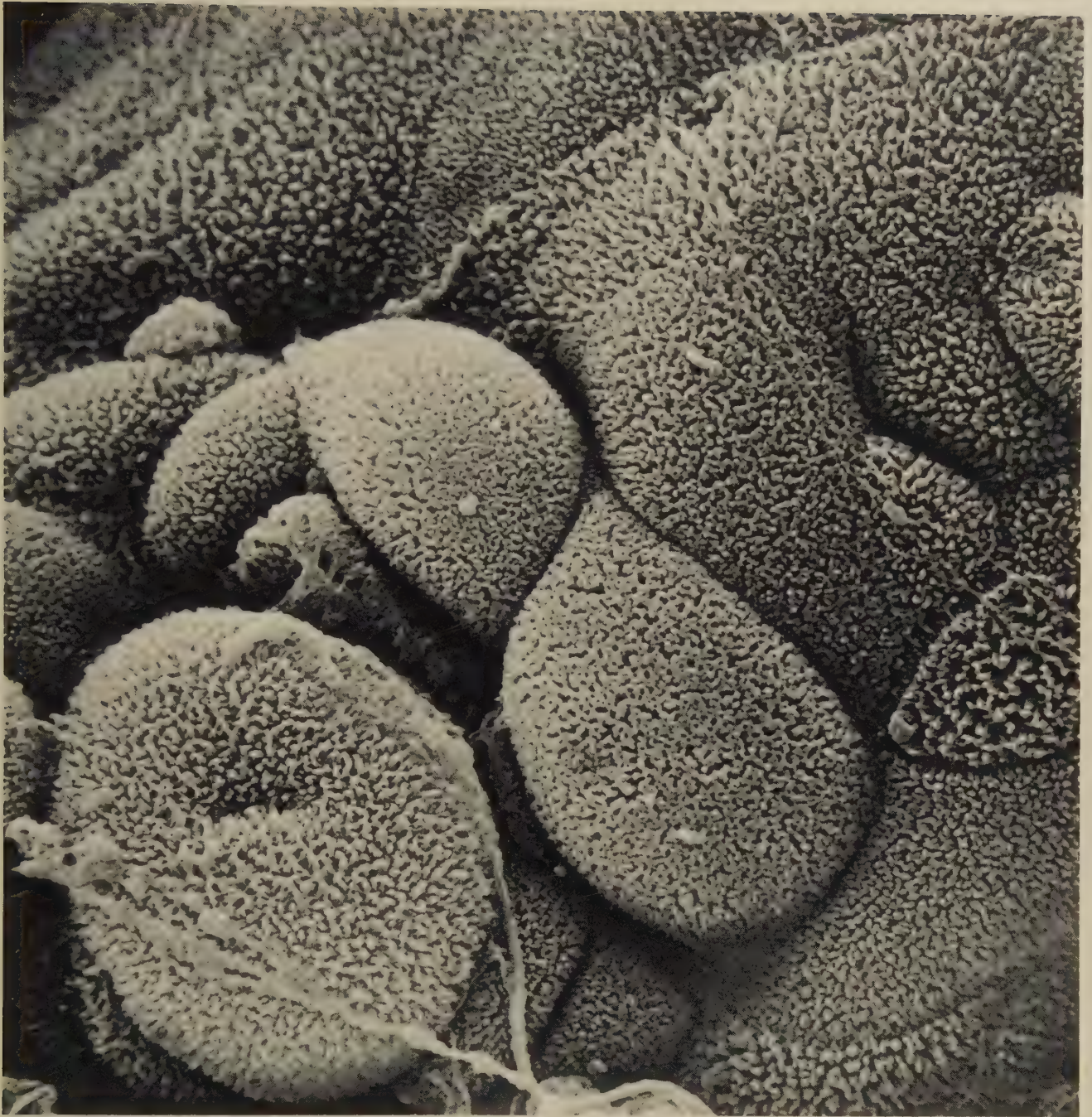


Fig. 126. Single dose 20 Gy – 3 days. Part of a villus top with various forms of cells and microvilli. On the top the cells are more swollen. 10,000 ×.

and have probably lost their function and now only keep the villi intact. Fig. 128 shows the different kinds of cells, extruding (a), cluster of separating cells (b), irregular and deformed flat cells (c). In higher magnification the separating cells are seen in

Fig. 126 with short, spread microvilli on the surface and in Fig. 127 all kinds of deformities are seen from long, narrow to circular, all with very short microvilli, which can be seen better in Fig. 131. There exists a difference between the top of the villus



Fig. 127. Single dose 20 Gy – 3 days. Drastic deformities of cells on the side of a villus. A long narrow cell in the center (a). Short sparse microvilli. 10,000 ×.

when extrusion is still going on and the side of the villus, as seen in Fig. 127. Cells on the side have marked boundaries and only short microvilli. No signs of mucinous granulae are detected and nothing like a goblet cell is seen at high magnification. The

number of cells per square unit is greater than normal (Fig. 127). On the flat villi no cells are extruded and the same cell may remain for a longer time. Beneath the surface there are only scarcely abortive crypts on LM.

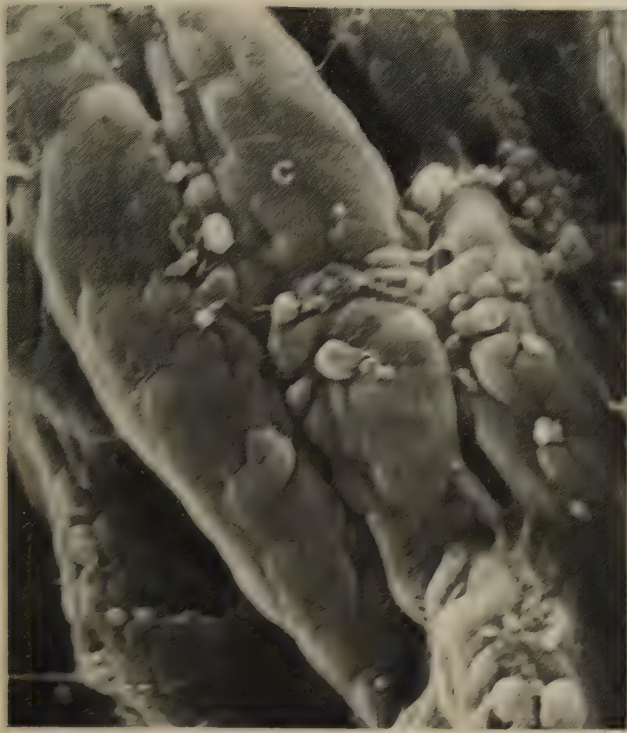


Fig. 128. Single dose 20 Gy - 3 days. Different types of cells on the slender, short villi. (a) extruding, (b) cluster of separating cells, (c) deformed flat cells. 4700 $\times$



Fig. 129. Single dose 20 Gy - 3 days. Irregularly formed epithelial cells on top of a villus. Short microvilli. 1900 $\times$



Fig. 130. Single dose 20 Gy - 3 days. Separating cells at high magnification. Spherical cell surface. Short sparse microvilli. 5500 $\times$

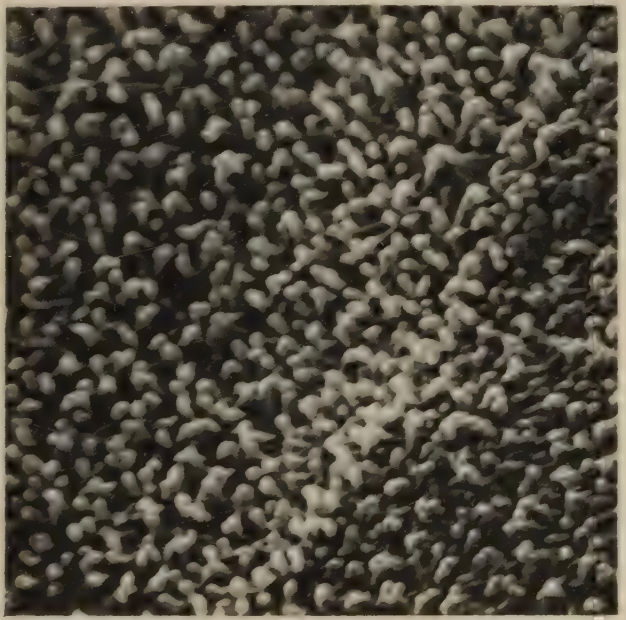


Fig. 131. Single dose 20 Gy - 3 days. Microvilli from Fig. 129 only 1/4 of normal height or even shorter with free surfaces between them on the cell. 11,000 $\times$

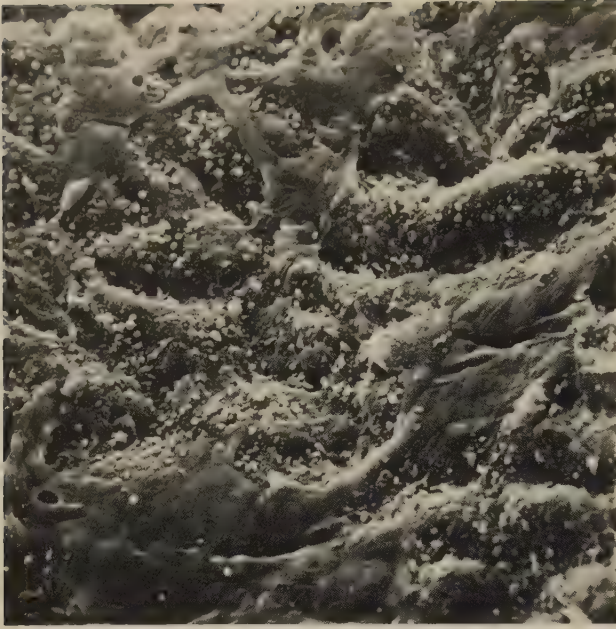


Fig. 132. Single dose 20 Gy – 5 days. Ulcerated surface. A thin layer of mucosa is left between areas where free cells are spread. Villi are only faintly hinted. Omega-cells seen in the lower part (a). 200 ×.



Fig. 133. Single dose 20 Gy – 5 days. In the lower part ulceration, in the upper part the epithelial lining is still there. 2000 ×.

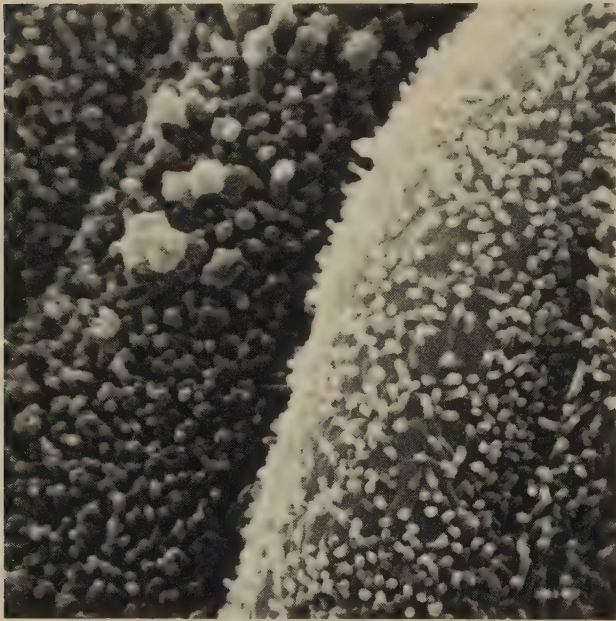


Fig. 134. Single dose 20 Gy – 5 days. The cells in Fig. 133 with scarce, very short microvilli. 10,000 ×.

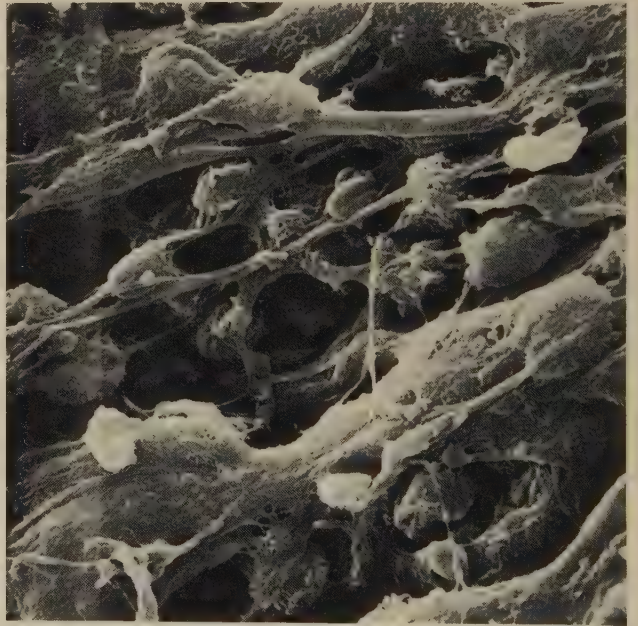


Fig. 135. Single dose 20 Gy – 5 days. The open ulcerated surface. Different kinds of cells. No bacteria. 2000 ×.

Single dose 20 Gy – 5 days

Two days later, five days after irradiation, only small upheavals are seen as remainders of the villi in a partly ulcerated mucosa. Fig. 132. Free cells are seen on the ulcerated surface and where the epithelial lining is still left, large polygonal omega cells have distinct boundaries. High magnification in Fig. 133 and 134, respectively, shows the open ulcer

and a few cells with remnants of microvilli as short plump outgrowths. It can be estimated that the quote between the area of mucosa and of ulceration is about 1:1. The situation is critical, as is registered even in the weight curve in Fig. 194, single dose, and gives a high score number on the arbitrary scale. Nowhere can any bacteria be seen, which is an important fact, with such a denudated or ulcerated open surface of the small intestine.



Fig. 136. Single dose 22.5 Gy – 5 days. Remnants of villi covered by an omega cell lining ulcerated in some places (arrow) with lots of free cells on the surface. No bacteria. 430 ×.

Single dose 22.5 Gy – 5 days

Five days after a single dose of 22.5 Gy the surface of the small intestine is almost flat. In Fig. 136 the earlier villi can be discerned, but most of the core seems to be destroyed and the covering epithelial lining is ulcerated (arrow). The omega cells are large and easily seen in Fig. 136(a) and in Fig. 137(a). Free

epithelial cells are spread here and there. Fig. 138 (arrow). There are no bacteria. The reaction is about the same as at 5 days after 20 Gy, (Fig. 132) and has been given the same score number. Fig. 139 is a high magnification of Fig. 136 at (b), where the irregularly formed and distributed cells have only a reduced number of microvilli per square micron, and as seen in Fig. 140, they are rudiments or very short.

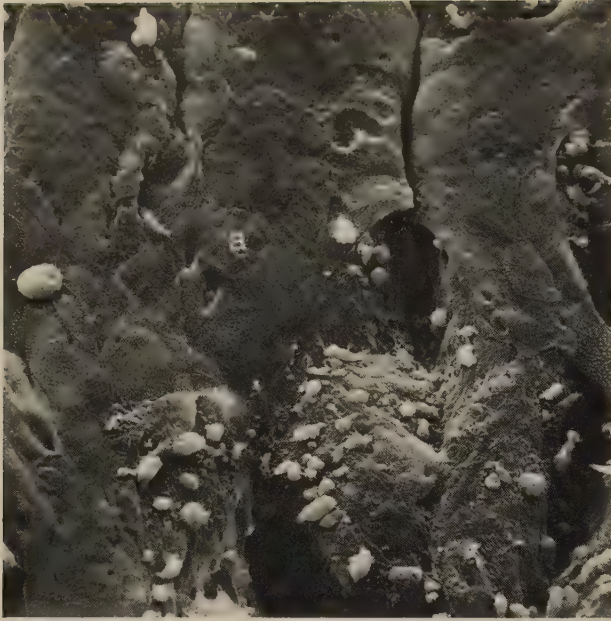


Fig. 137. Single dose 22.5 Gy – 5 days. An area in Fig. 136 with clearly seen omega cells and one ulcerated surface. 500 ×.

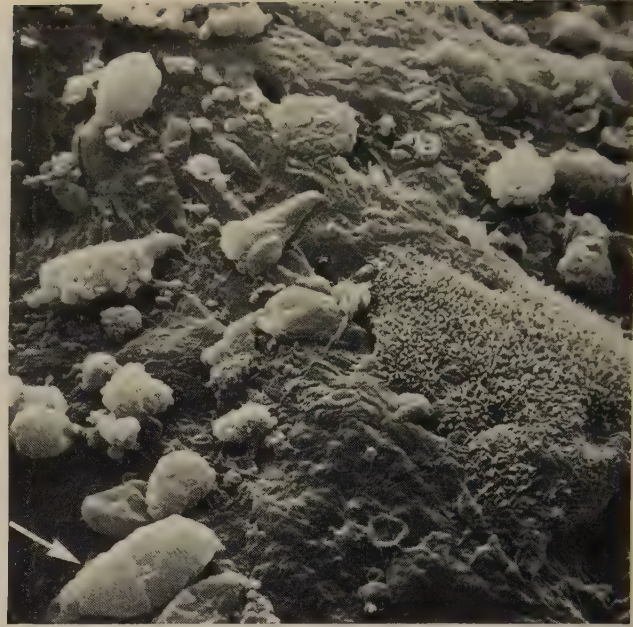


Fig. 138. Single dose 22.5 Gy – 5 days. Enlargement from Fig. 137 showing epithelial (arrow) and other cells on the ulcerated surface and a few remaining cells with microvilli. 2000 ×.



Fig. 139. Single dose 22.5 Gy – 5 days. Close-up picture of Fig. 136. Large, mostly irregular cells with well marked boundaries. 4300 ×.

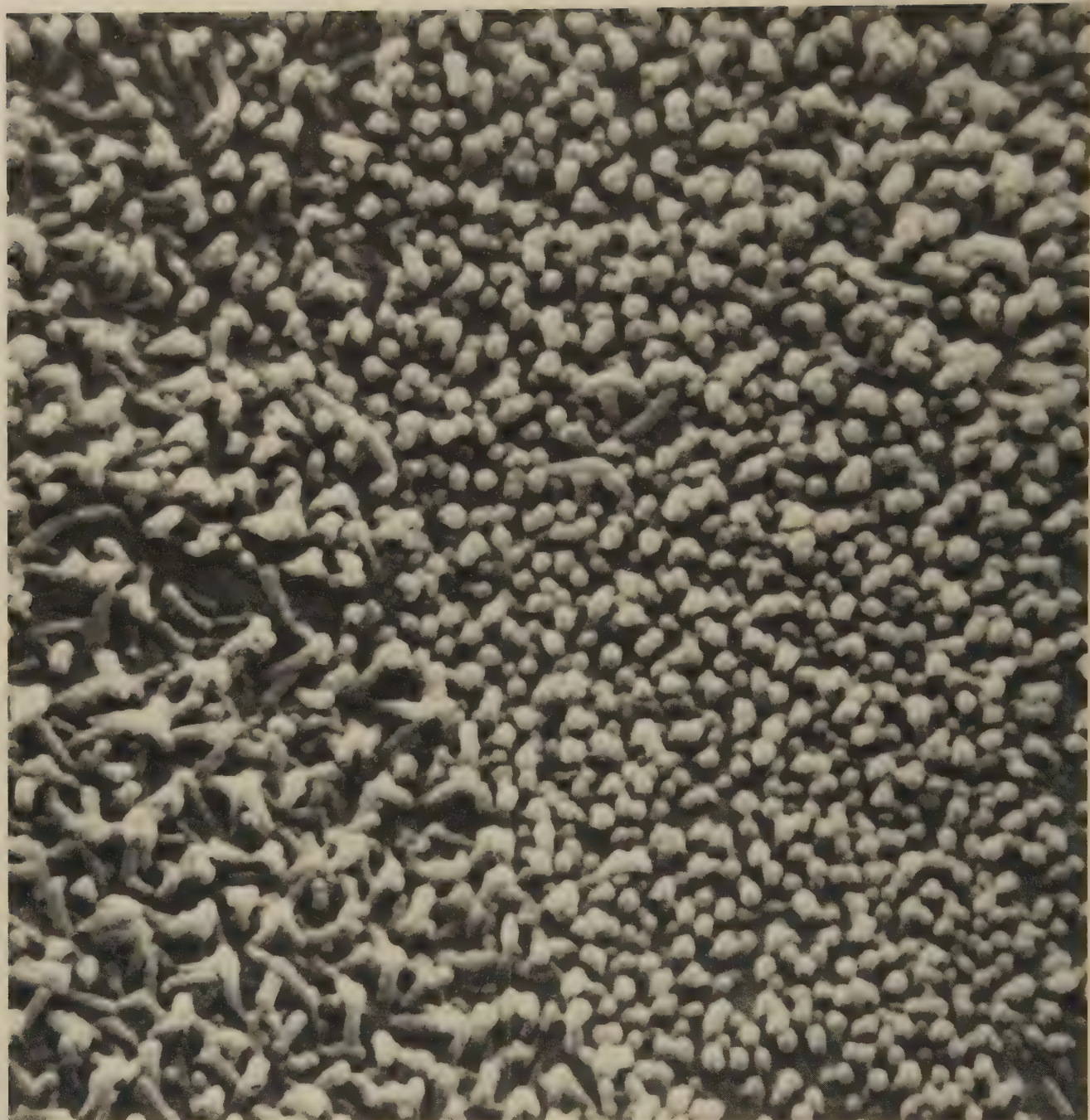


Fig. 140. Single dose 22.5 Gy – 5 days. Only the tips of the microvilli are left on the cell to the right. Magnification of part of Fig. 139. 20,000 ×.



Fig. 141. Single dose 22.5 Gy – 7 days. Completely flat surface. No remnants of villi. A residue of epithelial cell layer on the ulcerated surface. Omega cells are well marked and free cells are spread over the open section of the surface. No bacteria are seen. 1100 $\times$.

Single dose 22.5 Gy – 7 days

At 7 days after 22.5 Gy single dose, the reaction is still increasing with no signs of repair. The villi have completely disappeared as structures and the small intestinal mucosa are not far from total destruction. Only a minor area is now covered by epithelium. In Fig. 141 an extremely thin cell layer of omega cells polygonal in form is connected up to the right with

other intact epithelial remnants, but in the main the surface is ulcerated and open with free cells of different types, maybe penetrating from beyond. It is not possible to identify the tissue on the destroyed sections, Fig. 142. The horizontally large, but in depth thin, omega cells are flat and in close contact to each other with marked borders. In high magnification, as in Fig. 143, the microvilli on the omega cell-surface are so short that only a knobby



remnant resembling the top of the original microvillus can be seen. The diameter is about twice that of normal, $0.16\ \mu\text{m}$. In these pictures and in Fig. 141 there are no visible bacteria. Even after high doses the microvilli do not disappear. The cell surface is not destroyed. There are always microvilli, but fewer per square micron, and they are shortened to about $1/10$ of their normal length. In the highest magnification, it is not possible to identify any reaction or damage to the isolated microvillus.

Fig. 142. Single dose 22.5 Gy – 7 days. Part of the ulcerated area with some cells that might have come out from beyond the surface. 2000 $\times$.

Fig. 143. Single dose 22.5 Gy – 7 days. Knobby rests of microvilli, still with swelling of the tops. Diameter $0.16\ \mu\text{m}$. The picture is part of the surface of an omega cell. 21,500 $\times$.





Fig. 144. Single dose 25 Gy – 6 hours. A deserted landscape of swollen villi. 190 ×.



Fig. 145. Single dose 25 Gy – 6 hours. Retracted villi with folds. Crypt openings seen on the bottom between the villi. 470 ×.

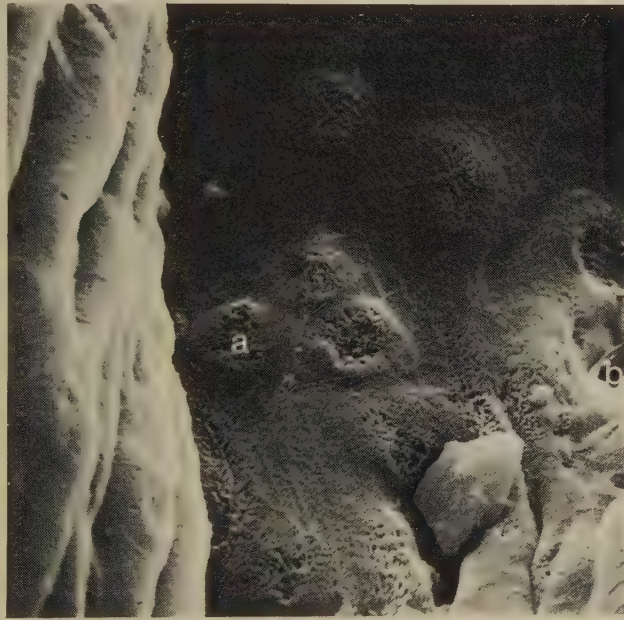


Fig. 146. Single dose 25 Gy – 6 hours. Bottom with Goblet cells (a) and crypt openings (b). 1900 ×.

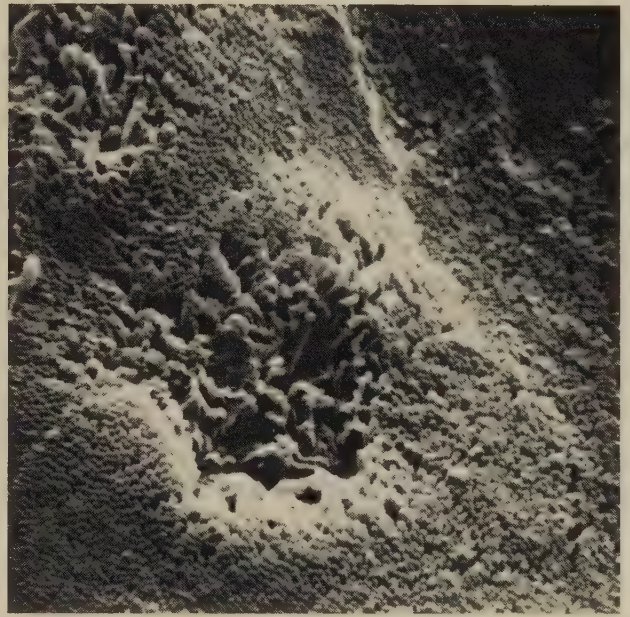


Fig. 147. Single dose 25 Gy – 6 hours. Goblet cells, non active. Normal microvilli. 9000 ×.

Single dose 25 Gy – 6 hours

The height of the villi is not notably changed from the retracted villi of normal eating animals. Villi are swollen and thus the folds are clearly depicted. There are no bacteria, but Goblet cells existing up to

the top. Maybe the rat is not eating, but the intestinal damage in function may have resulted in this oedematous but not active villi. No mucinous droplets can be detected. The epithelial cells are swollen and the microvilli almost normal.



Fig. 148. Single dose 25 Gy – 48 hours. Triangular villi. On the upper villus a group of cells is loosening. Closed Goblet cells. 1000 $\times$.

Single dose 25 Gy – 48 hours

The villi have a pyramidal form, a stage just before a shortening process through major extrusion at the narrow tops. Folds are well marked. Closed Goblet cells can be seen. There is no attack by bacteria. In Fig. 148 the villus in the upper part is rejecting almost all of its top cells. Only 24 hours later the villi

are very short, with clusters of cells still leaving each of the villi. The feedback mechanism, between the crypt and villus at 48 hours after the irradiation may therefore effectuate a dramatic change in the form of the villus, taking place during the next 24 hours.



Fig. 149. Single dose 25 Gy – 3 days. Short, elongated villi with a large amount of cells loosening at the tops. No bacteria. Sign of ulceration at the arrow. 430 ×.

Single dose 25 Gy – 3 days

At this dose about 70% of the rats die within 30 days (Fig. 191). In comparison with the result after 22.5 Gy there are great similarities in the appearance and structure of the villi. These are swollen, below half of the normal height and are long and slender. The pattern is moderately regular. Deep clefts separate

the cells on the upper section. On the extrusion zones there is a massive loosening of cells and no villus has the smooth top which can be seen at this stage at 20.0 and 22.5 Gy (cf. Fig. 125). This may be interpreted as a sign of a quick disappearance of the villi, which results in a flat surface (cf. *Fabrikant*

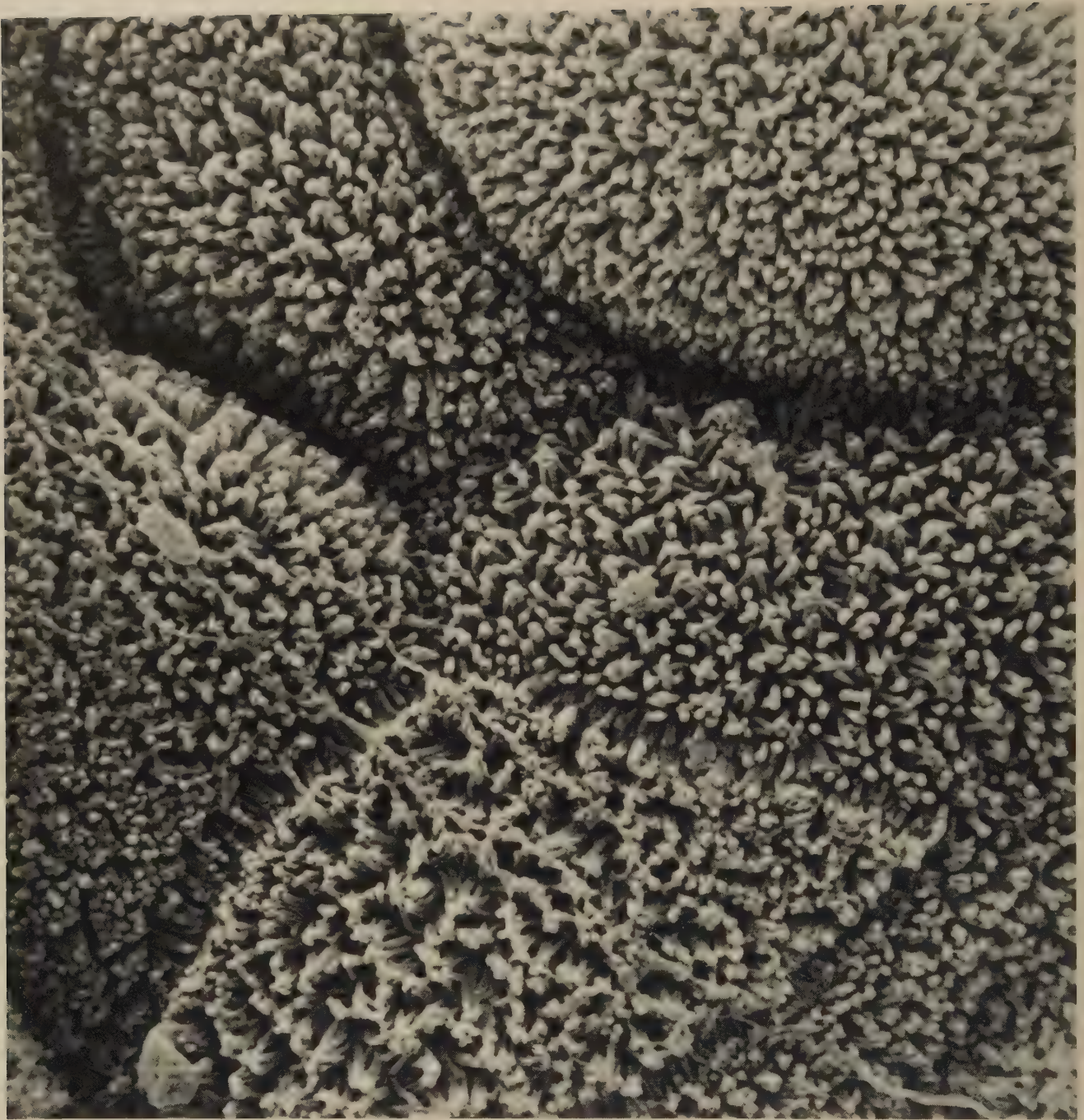


Fig. 150. 25 Gy – 3 days. High magnification of a villus with loosening cells at the top. The cell surfaces have different forms, are more or less spherical and the microvilli are spread with adherence between the tops. 5400 $\times$.

1972) and ulceration. At the arrow in Fig. 149 there is a sign of a suspected beginning ulceration. Fig. 150 shows at higher magnification the spherical epithelial cells on the top of a villus and the different sizes of the cells. The microvilli are diverging, but with groups piled up and with adherence at their

tips. In Fig. 151, part of Fig. 150 is seen at still higher magnification, which shows the club-formed end of the single microvilli. These are about one third of the normal length and have a diameter of 0.15 μm .

There are no signs of mucus droplets or Goblet cells. Bacteria are not present.

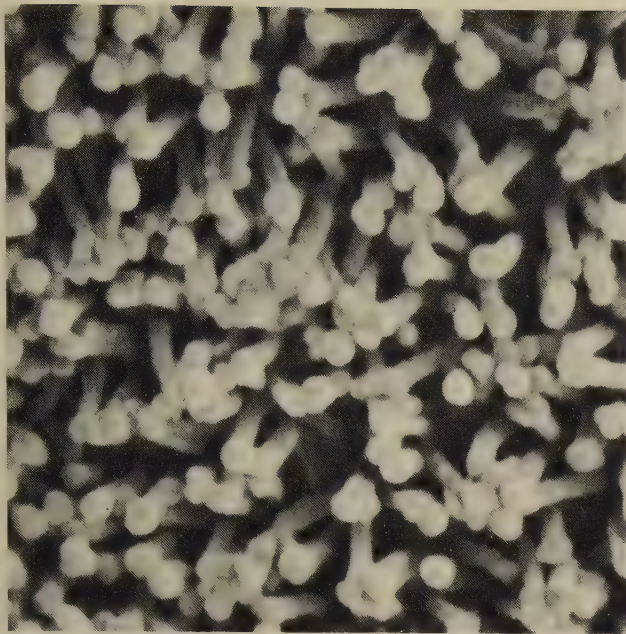


Fig. 151. Single dose 25 Gy - 3 days. Sparse distribution of microvilli with knobs at the ends. 20,000 $\times$.



Fig. 152. Single dose 25 Gy - 5 days. Flat surface with remnants of villi. Partly ulcerated. The thin epithelial layer is seen (arrow). 200 $\times$.



Fig. 153. Single dose 25 Gy - 5 days. Short microvilli, sparsely distributed on this cell surface, many of them are lying flat. 20,000 $\times$.

Single dose 25 Gy - 5 days

The villi have almost disappeared. Fig. 152. Ulceration has started and the thin epithelial layer with squamous cells is on its way to loosen (arrow). Islands of ulceration interfuse with rather intact mucosa. No bacteria are seen. Microvilli have shortened to about one-third of the normal height. They are sparse and most of them are lying flat. Fig. 153.



Fig. 154. Single dose 25 Gy – 7 days. Low magnification showing a large part of the intestine with intact sections of flat mucosa interfoliated with ulceration as islands within the mucosa. No bacteria. 400 ×.

Single dose 25 Gy – 7 days

The denudation is going on either to complete ulceration or to a stand still where repair can later on start building up new villi. In Fig. 154 about half of the surface is intact. The possibility of SEM-technique to get 3-dimensional documentation is seen here: islands of ulceration, big areas of ulceration

and also vast areas with flat but intact cell layers. No bacteria are seen, not even on Fig. 155, where a small residue of a couple of cells is located within a raw ulcerated surface with some free cells. Microvilli on the intact areas are very short but covering the cells. Fig. 156. The diameter of the top of a microvillus is twice the normal size, i.e. $0.16\ \mu\text{m}$.



Fig. 155. Single dose 25 Gy – 7 days. The rough surface with a few epithelial cells (arrow). Other free cells are spread over the naked area. 1100 $\times$.



Fig. 156. Single dose 25 Gy – 7 days. Microvilli in high magnification. Some are only knobs on the cell, others are bout 1/4 of the normal length. 21,500 ×.



Fig. 157. Single dose 30 Gy – 6 hours. Swollen, retracted villi with folds. Few cells in the extrusion phase. Goblet cells up to the tops. No bacteria. 470 ×.

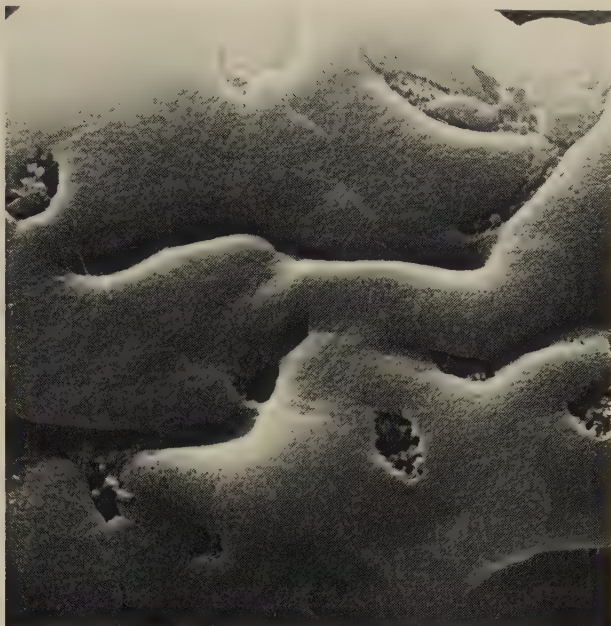


Fig. 159. Single dose 30 Gy – 6 hours. The epithelial surface is clean with no mucus granulae. 4700 ×.

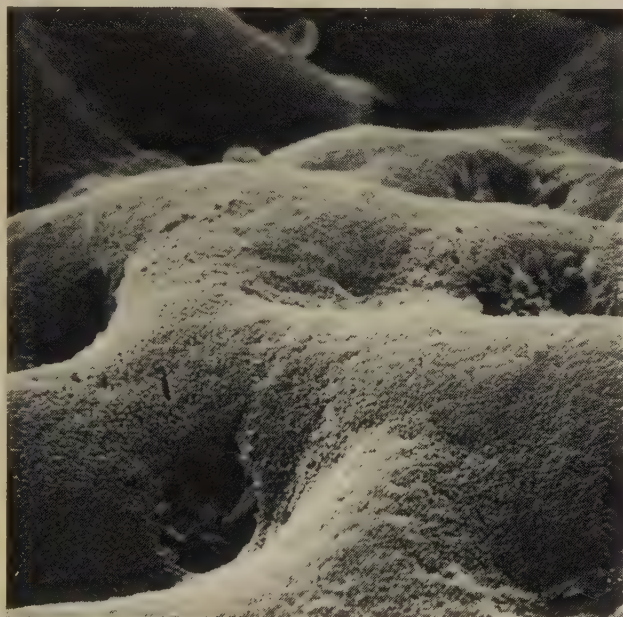


Fig. 158. Single dose 30 Gy – 6 hours. Enlargement of Fig. 157. The epithelial cells are connected to each other in a strict manner. 1900 ×.

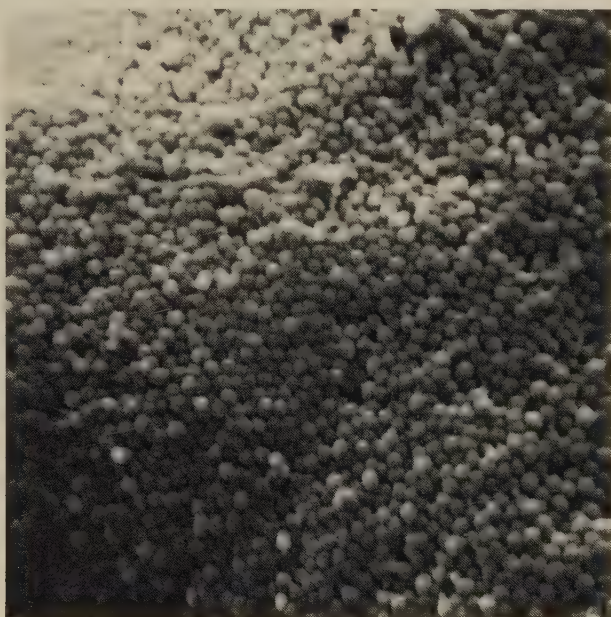


Fig. 160. Single dose 30 Gy – 6 hours. The same surface as Fig. 159 showing the microvilli closely packed, but not in the regular pattern as in a non-irradiated animal. 19,000 ×.

Single dose 30 Gy – 6 hours

The villi are swollen and retracted with distinct folds. The top of the villi has very few cells in the extrusion phase and Goblet cell openings are seen even at the top. They seem to be inactive and no mucus granula can be detected over the epithelial surface. The epithelial cells have a spherical form

and a smooth surface covered by a carpet of microvilli. The borderlines between the cells are tight and normal. The microvilli have not the normal regular pattern. The diameter varies, but is not far from normal (0.08–0.1 μm). No bacteria can be seen. Notable is the lack of bacteria and the non emptied Goblet cells all over the villus, which is as clean as in a fasting animal.



Fig. 161. Single dose 30 Gy – 3 days. Long, low, slender villi. No extrusion. Free cells on the surface. No bacteria. Starting ulceration at (a). Polygonal cells at (b). 400 ×.

Single dose 30 Gy – 3–5 days

This high single dose gives an even more increased reaction. The villi are long, slender and low, showing the long base of a villus. There is no longer any extrusion going on and a starting ulceration of the smooth epithelial surface can be seen, Fig. 161 (a). A lot of free cells are spread over the villi. No bacteria. In the low magnification isolated epithelial cells can be seen. (b) The situation can be explained as a further stage than after 25 Gy and 3 days. The remarkable loss of cells is over and only 1 day later the villi will have broken down to a plain surface

with a thin cell layer. Thus, the increasing effect of different single doses registered at 3 days seems not only to be related to dose, as is presented in the score-curves in discussion, but also to time. – The surface after 3 days is covered by flat, mostly polygonal epithelial cells, Fig. 163, where the border is distinct and the microvilli are short and reduced per square micron. The length is about $0.4\ \mu\text{m}$ and the diameter at the top twice that of normal. The distance between MV is such that the cell-surface on which the MV stand can be seen. The top is club-formed.

Fig. 162. Single dose 30 Gy - 3 days. Cells from the villi in Fig. 160. Different kinds of forms. Distinct borders. 1900 $\times$.



Fig. 163. Single dose 30 Gy - 3 days. High magnification from Fig. 160. Flat cell surfaces with clear borders. MV with swollen ends, scarcely distributed so that the cell membrane can be seen between them. 10,000 $\times$.



Fig. 164. Single dose 30 Gy – 5 days. Ulcerated surface to the left and intact epithelial layer to the right. Free cells on the open area. 1000 $\times$.

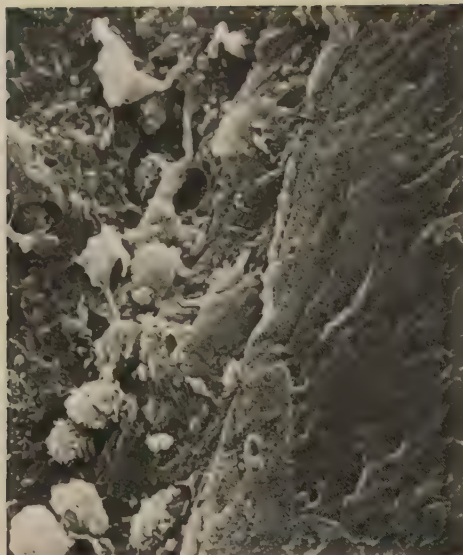


Fig. 165. Single dose 30 Gy – 5 days. High magnification of Fig. 161. Intact cell layer. Variation in cell form. The cells surrounding the opening have better preserved microvilli. 4300 $\times$.



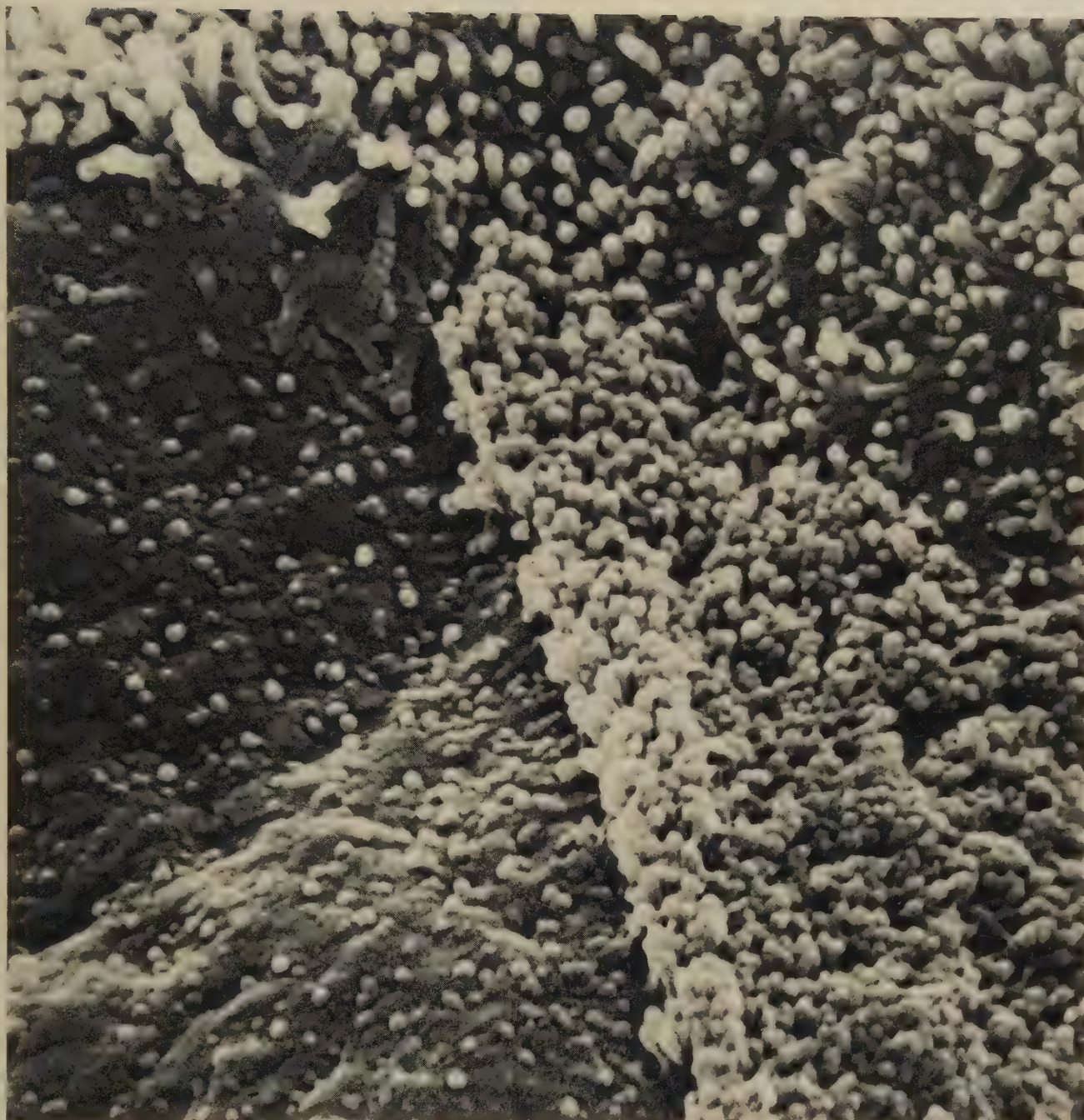


Fig. 166. Single dose 30 Gy – 5 days. Two cells with different residues of microvilli. Only a few tops on the surface to the left. 11,000 ×.

After 5 days the intestinal surface is ulcerated in the same way as was seen after 22.5 Gy and 25 Gy at 5 days. Only small sections of the irradiated ileum have a thin layer of omega cells. The normal epithelial cells are described as hexagonal, but after irradiation the squamous cells take any various forms from small and narrow to circular and polygonal. As demonstrated after other high single doses the ulcerated surface has free cells on it, maybe coming from underlying tissue. In Fig. 166, a magnification from Fig. 165, even the microvilli seem to be damaged. The cells surrounding the opening, which can be a crypt opening, have a rather

tight carpet of very short microvilli. The other cells are almost totally depleted from microvilli. This has not been seen in lower doses at any time. Instead the surface usually ulcerates, with cells having an even distribution of short MV. Fig. 166 illustrates the described differences on cells. To the right there are short MV but on the cell to the left only a few tops of microvilli are left on an otherwise empty cellmembrane. The diameter of a top of a microvilli on both cells is between two and three times that of normal. No other dose has shown such swollen and enlarged microvilli. A constant diameter of twice that of normal has usually been found after irradiation.

Repair

Single dose 15 Gy – 14 days

Fourteen days after a single dose of 15 Gy, the surface structure may be interpreted as a repair process. The regular pattern of raised parallel ridges is not seen, but individual, convex structures have grown up. The situation of the ileum is judged as a score number (1.0), being aware of the fact that a real comparison with a score in the destruction

phase is not fully correct. The repair process is also reflected in the weight curve of the animal (Fig. 190) since the decrease in weight has changed to an increase.

The surface of the new villi structures is smooth, and the flat epithelial cells are covered with microvilli, some of which have club-like swellings at their tips. The Goblet cell openings can be seen right up to the top of the villus. No emptying has taken place and there are no cells in extrusion phase. No bacteria are seen.



Fig. 167. Single dose 15 Gy – 14 days. Villi in the repair phase growing up irregularly, some of them horse-shoe formed. 100 ×.



Fig. 168. Single dose 15 Gy – 14 days. Enlargement from Fig. 167. New short microvilli in an irregular pattern. 5000 ×.

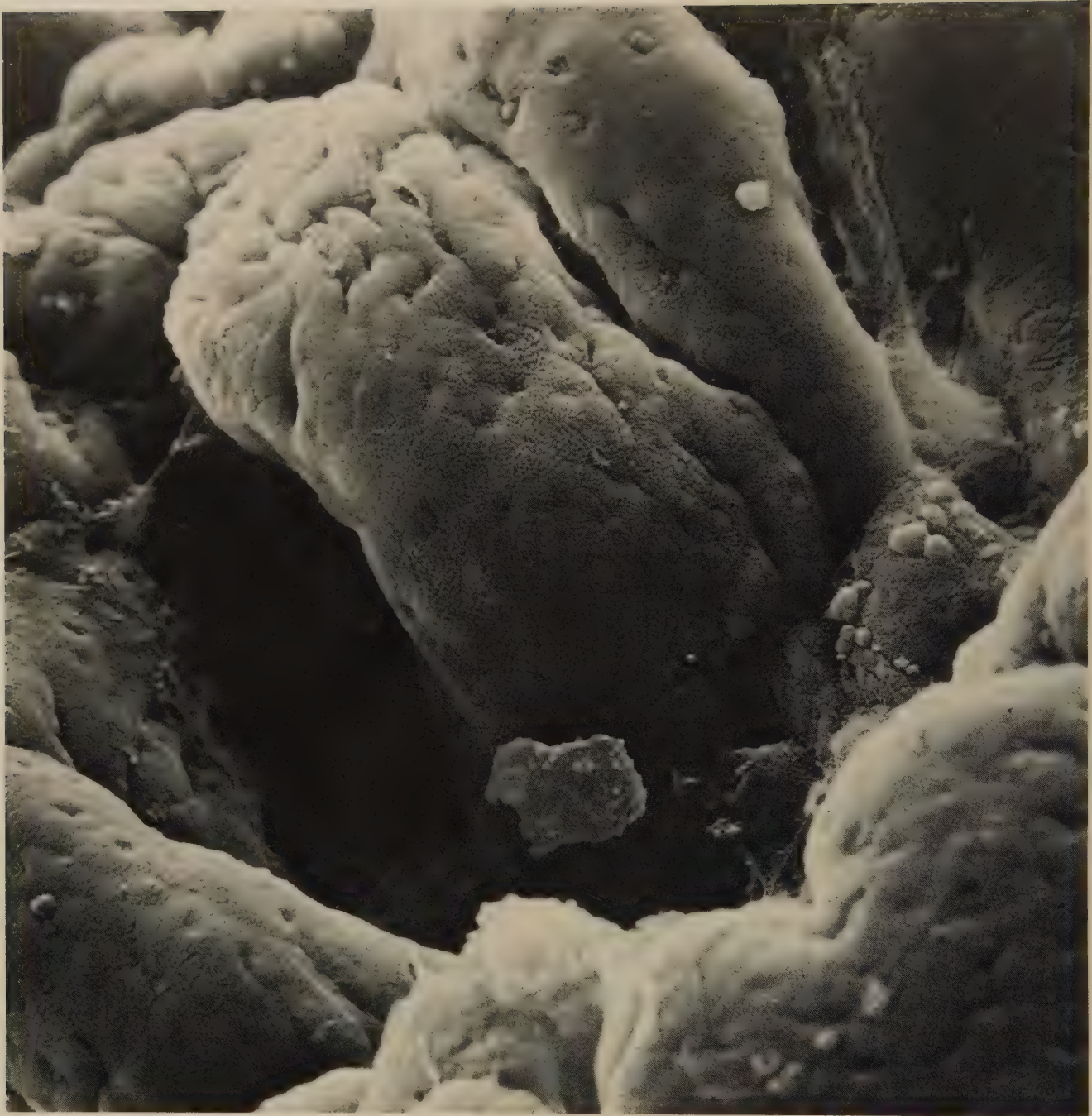


Fig. 169. Single dose 15 Gy – 14 days. Enlargement of Fig. 167. In the center one large growing villus. Plain surface. Closed Goblet cells. No bacteria. 1100 ×.



Fig. 170. Single dose 20 Gy – 14 days. Regeneration of villi. The arrow shows the new formation further enlarged in Fig. 172. Openings of many Goblet cells – not functioning – are seen (enlarged in Fig. 171). 430 ×.

Single dose 20 Gy – 14 days

The healing process after the administration of 20 Gy goes on slowly and still 14 days after the irradiation, the mucosa is not at all restored. Some rats have died. It can therefore be assumed that those rats who survive don't show full healing since the dose is very near the $LD_{50(30)}$ (22.5 Gy). Some villi have grown up, not to the normal appearance, and others are on their way to shape villus-like

structures, which is shown in Fig. 170 (arrow). The normal strict order and arrangement of the villus structure is far from the appearance before the irradiation. On the intestinal surface as a whole there exists an irregular pattern of a bulky mass of cell assemblies supposed to be the final functioning villi. In comparison with the destructive process in the early phase of the experiment the situation of the mucosa is scored to around 2 in Fig. 186. This is

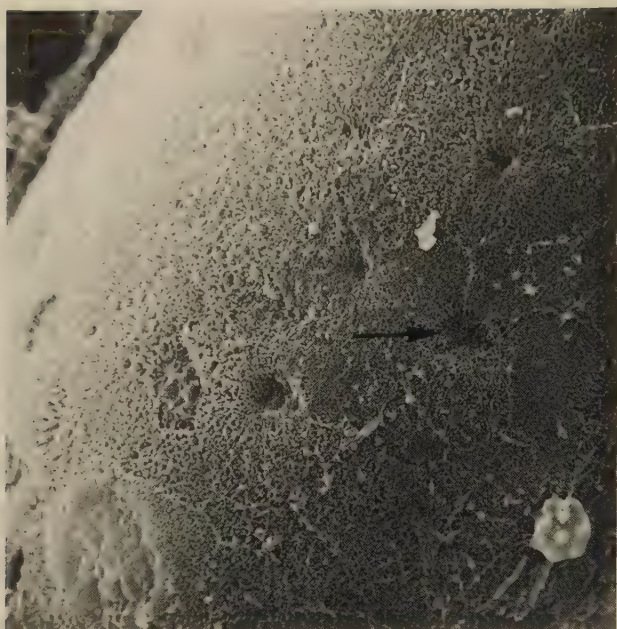


Fig. 171. Single dose 20 Gy – 14 days. Smooth surface on a villus. Closed Goblet – cell openings (arrow). 2000 $\times$.

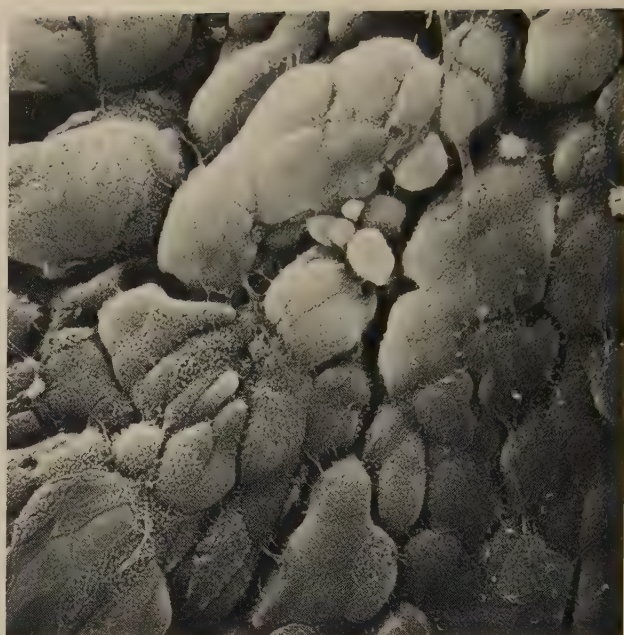


Fig. 172. Single dose 20 Gy – 14 days. Enlargement of part of Fig. 170. (arrow). The vertex of a villus growing up with groups of spherical epithelial cells. 1000 $\times$.

shown in Fig. 186 as an open circle, since the viewers were aware of the two situations and that there is so far only a similarity with the destructive process. The healing is further underlined by the existence of many Goblet cells, intermingled between the epithelial cells, which have a flat surface and polygonal form and closed borderlines (Fig. 171). The Goblet cells show no sign of activity. There is no real extrusion zone, but even the small rudimentary villus-top (Fig. 172) shows spherical cells and deep clefts. The extrusion has not been scored at 14 days after irradiation. Microvilli cover the epithelial cells (Fig. 173). The appearance of these microvilli is not far from those seen in the destruction phase. Some cells have an area covered by short symmetrically arranged microvilli. Other cells have clumsy microvilli which adhere together at the top. This phenomenon is also seen in the destruction phase (cf. Fig. 124). The phenomenon of a swelling of the tips is striking. The difficulties in judging the surface are reflected in the judging of the score number of 2–2.5 for epithelial cells and 1.5 for the microvilli (Fig. 186 and 187). Bacteria are not seen at this time after irradiation.



Fig. 173. Single dose 20 Gy – 14 days. Enlargement of part of Fig. 172. showing the covering of epithelial cells by microvilli. Great difference in the microvilli structure and arrangement is seen. 5000 $\times$.

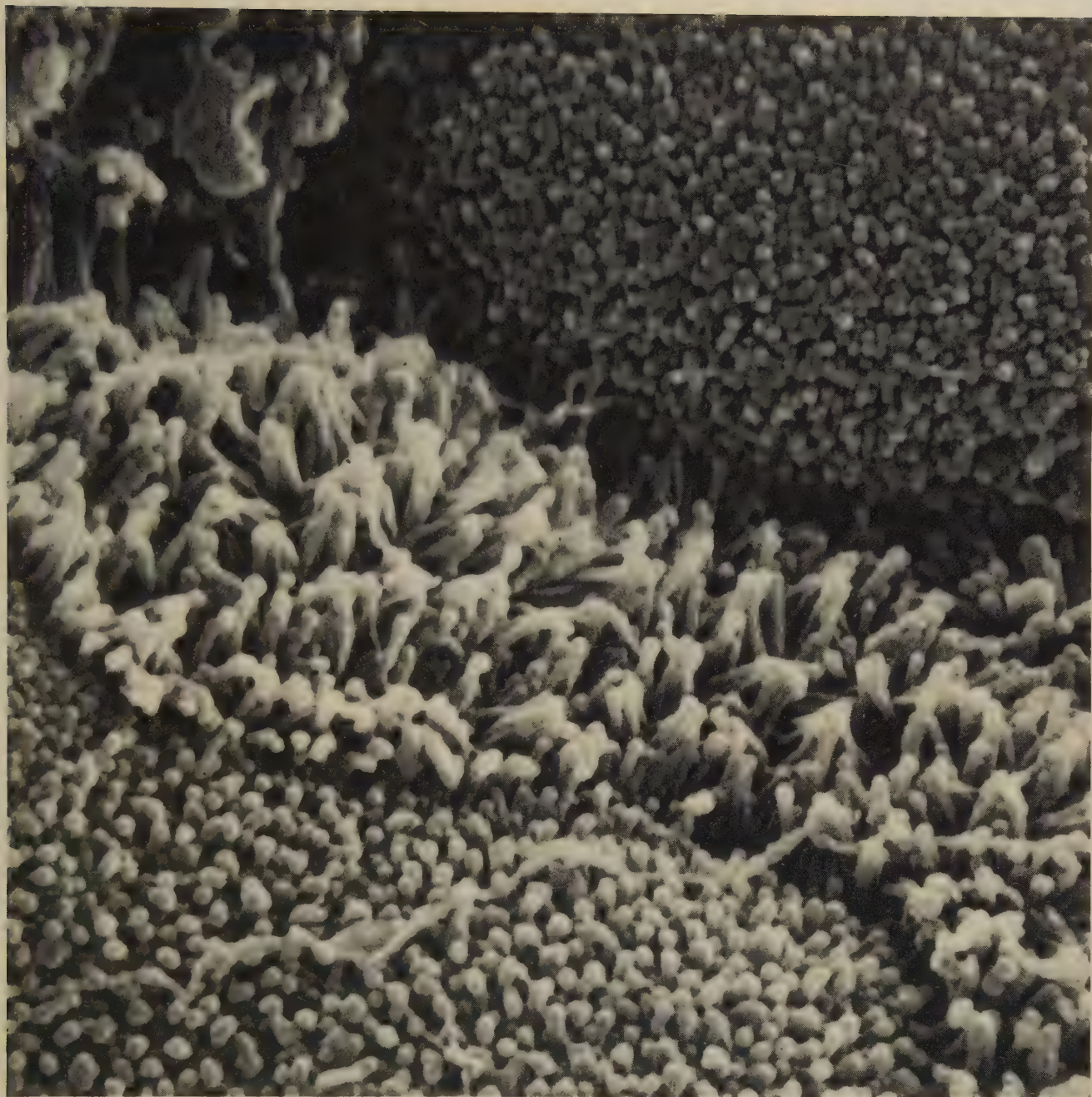


Fig. 174. Single dose 20 Gy – 14 days. Further enlargement of part of Fig. 173. Three epithelial cells are seen with different patterns of microvilli from tightly packed to widely spread with adhesive tips. Most of the microvilli are swollen at the tips (diameter about $0,2\ \mu\text{m}$). $10,000\times$.

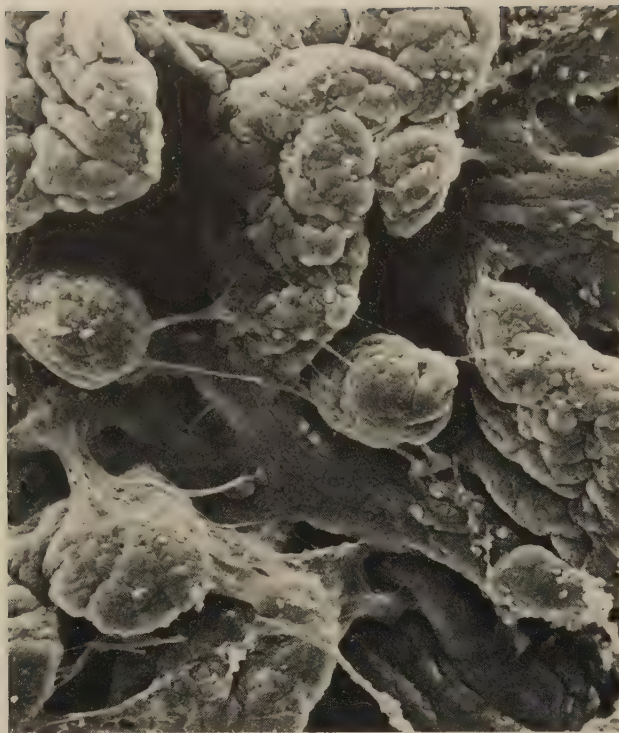


Fig. 175. Single dose 20 Gy – 21 days. Finger-like or pyramidal villi. Folds and traces of extrusion. 130 ×.

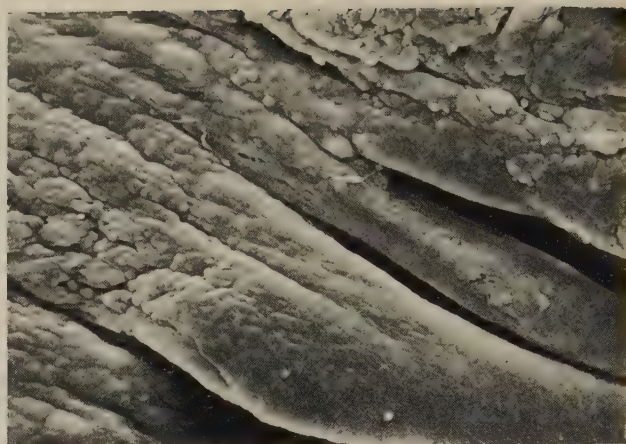


Fig. 176. Single dose 20 Gy – 21 days. Developing villi with a smooth surface on the side and an accumulation of irregular cell-clusters at the middle part. 200 ×.

Single dose 20 Gy – 21 days

Three weeks after irradiation the regrowth of villi has progressed, and it is possible to discern individual villi, erected but low and irregular. Folds have been developed on the sides and many have a pyramidal shape (Fig. 175) like those in the destruction phase, which were judged as a retraction phenomenon. Now, during the new-forming phase, the same appearance may indicate for another explanation, e.g. the building up of the marrow in the center with arteries, veins and lymph-vessels. There is a spectrum of different degrees of renewal: low smooth ridges with traces of an accumulation of spherical cells on an extrusion zone (Fig. 176). The villi have a more or less well-defined ones extrusion zone. The epithelial cells, both on the low villi and the more developed, have attained a normal appear-

ance with tight borderlines and a polygonal form (Fig. 179, arrow). Even on the smallest ridges the microvilli are seen to be normal (Fig. 178), although the normal arrangement is not found. The diameter of the microvilli is about $0,1 \mu\text{m}$. Goblet cells have now reached the normal number, but their functional activity has not been established. The opening of a Goblet cell looks normal (Fig. 178), prepared to empty its content.

Scoring the surface from the appearance of the villi, epithelial cells and microvilli, is easier than at the time 14 days after irradiation. The structures have been given the score numbers 0.5–1.0.

No bacteria are present and the functional capacity of the ileum has not come to the normal level, as judged from these pictures. The increase in weight speaks for return to normal repair-activity.



Fig. 177. Single dose 20 Gy – 21 days. Enlargement from Fig. 176. Epithelial cells and Goblet cell openings. 2000 $\times$.

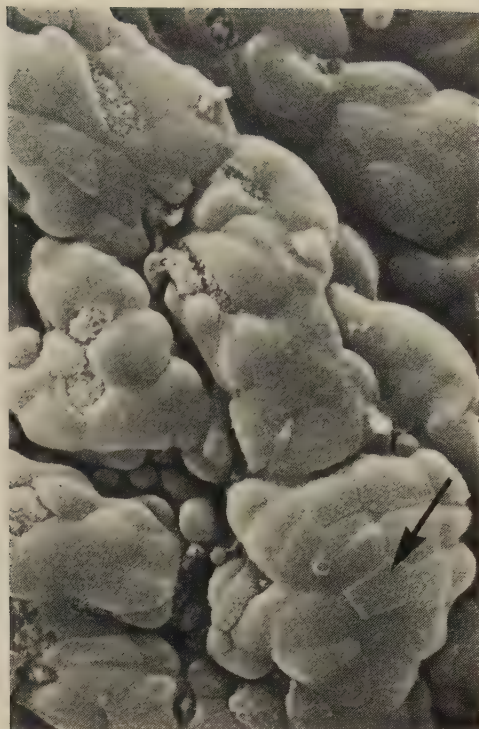


Fig. 179. Single dose 20 Gy – 21 days. New villi growing up with well demarcated polygonal cells (arrow). 1000 $\times$.



Fig. 178. Single dose 20 Gy – 21 days. Enlargement of Fig. 177. Goblet cell with closed opening. 26,000 $\times$.

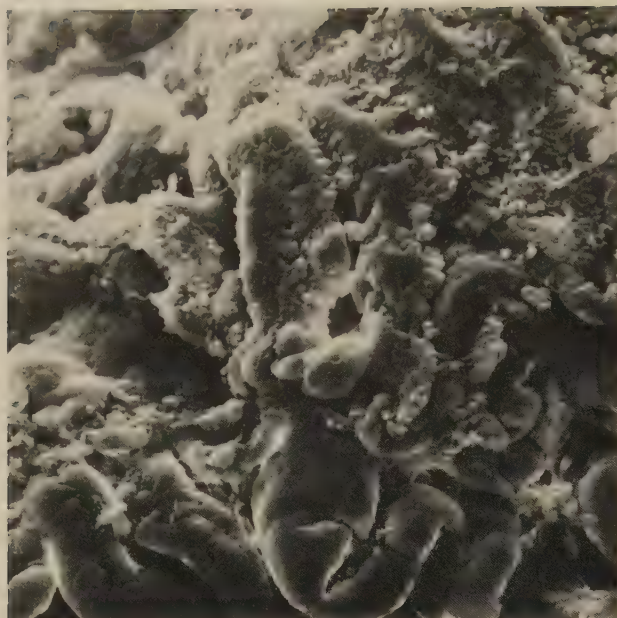


Fig. 180. Single dose, 22.5 Gy – 14 d. Surface of the ileum. In the upper part of the picture ulceration is seen. In the lower part new villi are being formed. 190 ×.

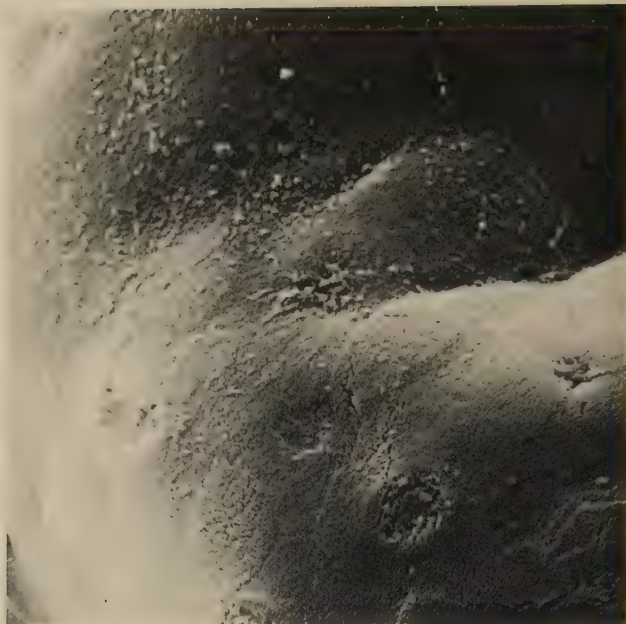


Fig. 181. Single dose, 22.5 Gy – 14 d. Enlargement of part of Fig. 180. Goblet cell-openings are seen and the villus covered by regular smooth epithelial cells. 1900 ×.

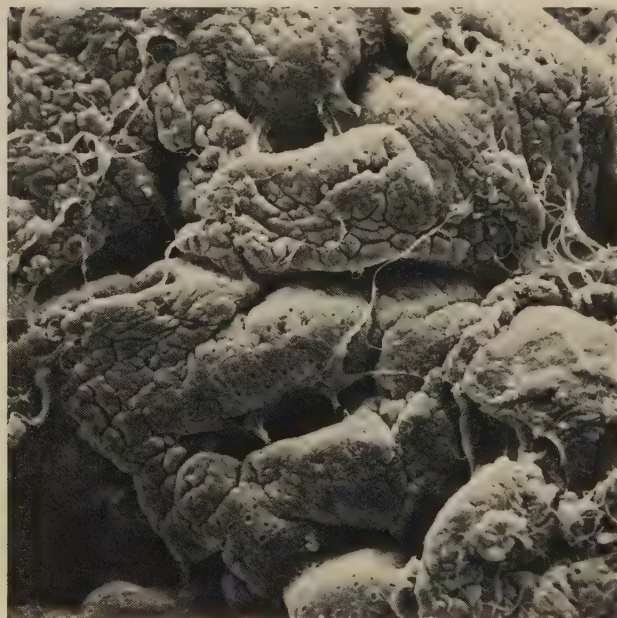


Fig. 182. Single dose, 22.5 Gy – 21 d. Irregular pattern of newly formed villi with Goblet cells on the surface and solitary bacteria. 190 ×.



Fig. 183. Single dose 22.5 Gy – 21 d. Enlargement of part of Fig. 182. Bullous structures covered by epithelial cells with microvilli. One bacterium is seen in the upper part.

Single dose 22.5 Gy – 14 days

22.5 Gy is the $LD_{50(30)}$ in these experiments. The situation is critical for the animals. The repair process is going on. The surface has ulcerations and denuded villi. (Fig. 180), but new villi are forming with flat, normal epithelial cells and Goblet cells

with closed openings. The activity of these cannot be seen (Fig. 181). The score number for the villi is therefore higher than for 20 Gy (1.5–2). The epithelial cells appear as normal on the villi-like structures and as disintegrated cells on other places. In fact it is impossible to say if we have a continuous destructive process or delayed destruction. It may



Fig. 184. Single dose 22.5 gy – 21 d. Another part showing folds, Goblet-cell-openings and well-marked borderlines between the epithelial cells. 1900 ×.

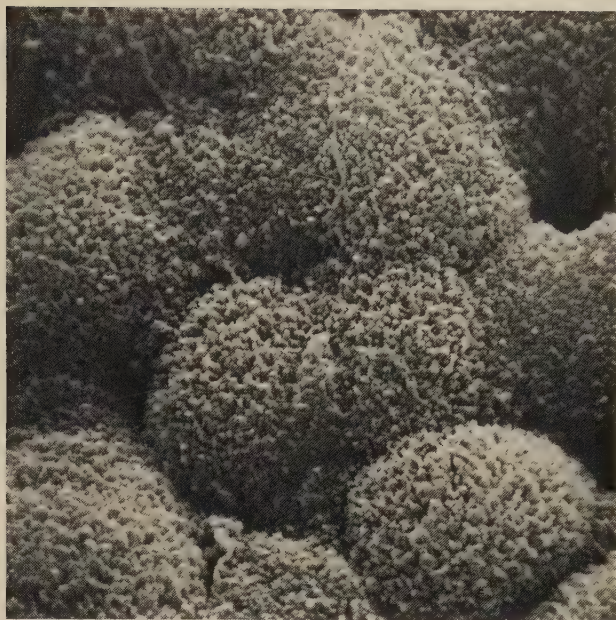


Fig. 185. Single dose 22.5 Gy – 21 d. Enlargement of Fig. 183. Villus from the side. 4700 ×.

be a balance between both and since repaired normal epithelial cells are seen, the score number is judged as 2.0. At 14 days no bacteria is to be found. The microvilli on the developed epithelial cells (not shown in the figure), are very similar to those seen 14 days after 20 Gy and are given the score number 1.5. The physiological activity of such a surface may by necessity be bad and this is reflected in the steady decrease of the weight of the animal.

Single dose 22.5 Gy – 21 days

On the surface of the gut, irregular protrusions of villi-like structures have grown up (Fig. 182). The normal arrangement is not seen. The villi have a

rather bullous character and have gained a height of about 50% of the normal one. A rich amount of Goblet cell openings are seen and the villi seem to be totally covered by epithelial cells.

No denudation or ulceration is seen. The epithelial cells are scored to 1. They have a more or less spherical form (Fig. 183). The surface of the epithelial cells is covered by a carpet of low microvilli. These have not the normal arrangement. The diameter is normal but many of these microvilli have vesicles at the tip. They are scored to 1.5. No completed extrusion zone is seen. A normal tendency to functional activity is supposed to occur at this time since bacteria can be detected (Fig. 182). The normalization is further emphasized by the weight increase (cf. p. 137).

Score curves

Score curves

The reaction of the intestinal surface after single dose irradiation is followed at certain intervals: 1, 2, 3, 5, 7, 14 and 21 days and the score numbers of each dose are plotted in Fig. 186 (villus), in Fig. 187 (epithelial cell) and in Fig. 188 (microvilli). These three different structures are scored individually after all doses although they together compose the entity of the villus.

The average reaction is scored on an arbitrary scale against time after irradiation, from 0.0 to 4.0, where 4.0 is defined as ulceration. The results summarized may be divided in three phases.

Phase I is the period where almost no reaction is seen morphologically and where a kind of state of stimulation can be seen in low doses and in high doses initially. At the same time, during the first 48 hours, damage to the crypts means a dramatic reaction below the surface, not seen by SEM. It takes more than 48 hours before most of the given single doses result in any change of the villi. In this phase no difference is seen between low and high doses in the average reaction.

Phase II can be said to be the destructive period of the mucosal surface. Starting at day 3 the spread in score number is obvious, dependant on the size of the dose. Low doses, 6–12 Gy, have a maximum

of mucosal reaction after 3 days and turn to repair at 5 and 7 days. High doses, 20–30 Gy, increase in reaction until a maximum at day 7. The difference in score number is small. All four single doses, 20, 22.5, 25 and 30 Gy, destroy the villi and end up in a more or less ulcerated, plain, omega cell covered surface.

The dose with a sequence of score numbers between low and high is 15 Gy. The maximum reaction is 1.5 in score after 3 days and no change is recorded after 5 and 7 days. From Fig. 186 and also from the weight curve in Fig. 190, 15 Gy can be seen to constitute a kind of a borderline dose. In the LD₅₀₍₃₀₎ experiment almost 100% of the animals given 15 Gy were alive after 30 days.

Phase III is the period of death or repair. At day 7 animals given low doses, 6–12 Gy, villi are already repaired and functioning. 15, 20 and 22.5 Gy as shown in the section of repair, result in a slow process of regrowth of the villi which is not finished at day 21. At these doses most crypts have disappeared and only a few cells are left. The proliferation is seen to produce an irregular pattern of new villi at day 14. It has not been considered possible to use the score system in phase III but still open circles are plotted in the figures roughly showing the state of repair. A major part of the animals given 25 Gy and all given 30 Gy die in extensive

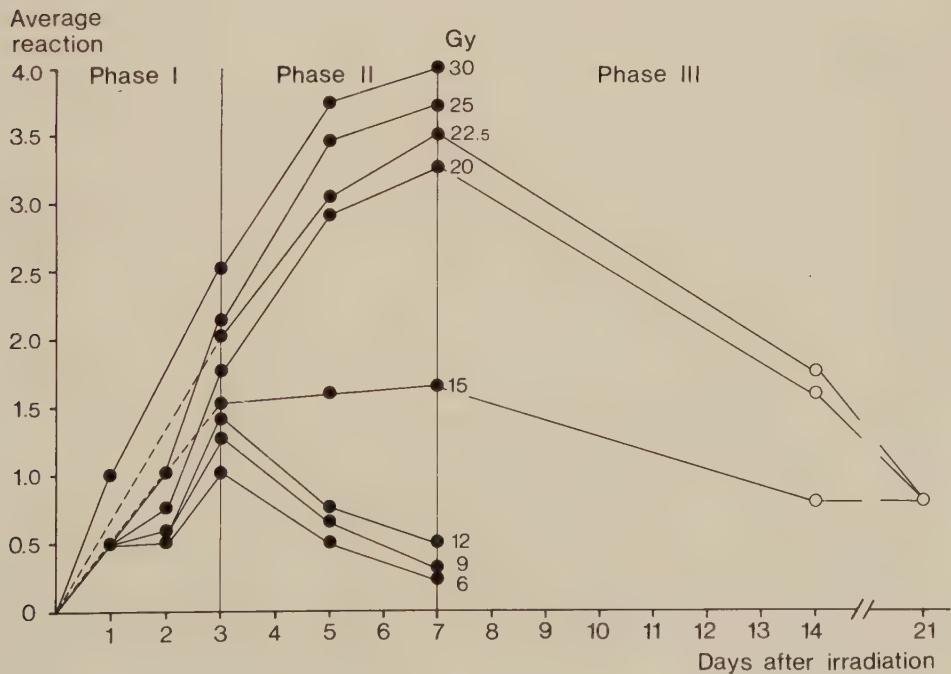


Fig. 186. Single dose irradiation. Average reaction scored on an arbitrary scale. Villus-reaction at different doses and days after irradiation.

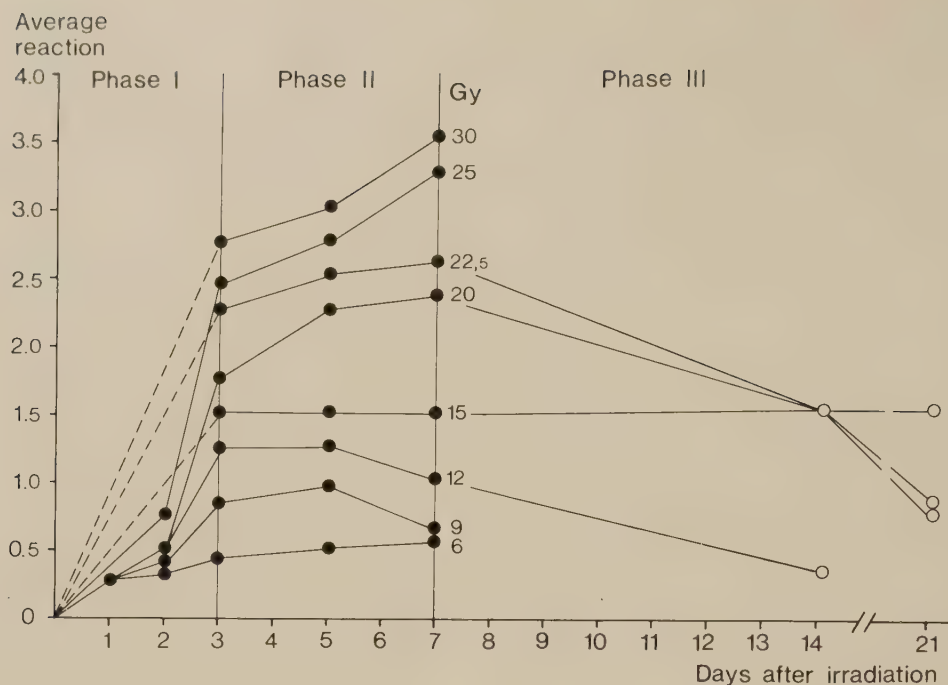


Fig. 187. Single dose irradiation. Average reaction scored on an arbitrary scale. *Epithelial cell reaction* at different doses and days after irradiation.

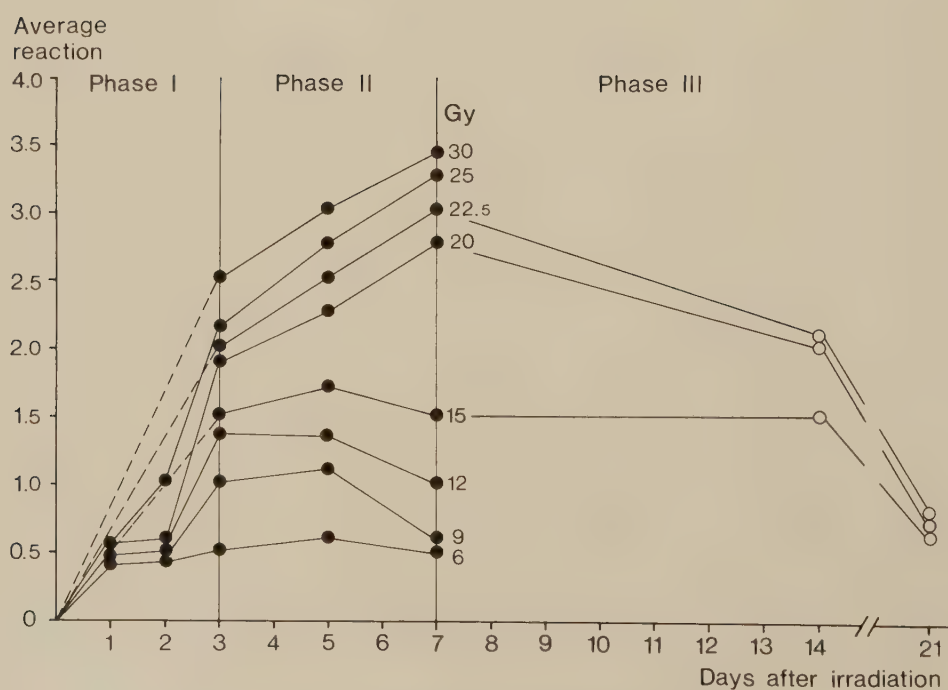


Fig. 188. Single dose irradiation. Average reaction scored on an arbitrary scale. *Microvilli reaction* at different doses and days after irradiation.

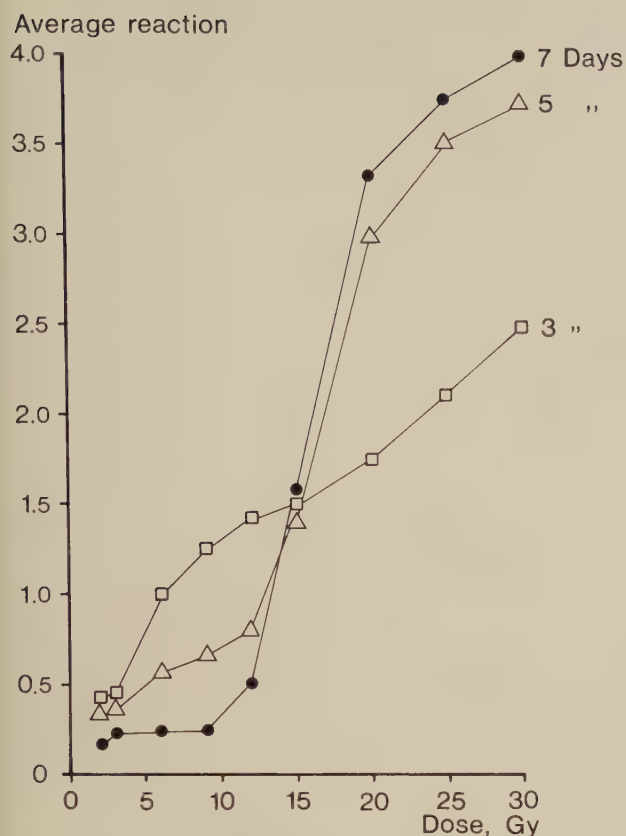


Fig. 189. Single dose irradiation. Average reaction scored on an arbitrary scale. Total reaction of the ileum mucosa at 3, 5 and 7 days after different doses. The reaction scored at the dose of 15 Gy is the same at these three points of time after irradiation.

or total ulceration with no repair being possible before other factors are killing them. The long period of time before restoration of at least 50% of the height of the villi is in line with the critical period of the weight between day 10 and 21, as seen in Fig. 194, probably showing a not yet functioning intestinal mucosa. SEM clearly demonstrate the difference in reaction between a single dose of 15 Gy and 20 Gy. After 15 Gy the villi are reduced in height to no more than about 50%. After 20 Gy the villi are reduced to zero. Still repair is slow in both cases. After 15 Gy the crypts are destroyed and the stroma or the core may also be damaged to a degree making the process of regrowth hardly different from the situation after 20 Gy, where no villi are left after 7 days.

In Fig. 189 the average reaction at the different single doses is plotted at day 3, 5 and 7 after irradiation. The constant score number of 1.5 at 15 Gy is a crossing between low and high dose reaction. The sharp increase in the average reaction after 20 Gy between day 3 and 5 is illustrated as well as the small difference between day 5 and 7. The disappearance of the villi takes place in the short time time of 48–72 hours.

Body weight

Body weight

Changes in body weight following irradiation have been shown to be a reliable and sensitive sign of the irradiation effect in damage as well as in repair (Smith and Tyree, 1954; Conard, 1956; Sténson, 1969; Taketa, 1962). Change in function and morphology of the intestinal mucosa is reflected in the body weight. Because SEM technique shows the mucosa together with some of their functions, it has been considered of value to control the weight during and after irradiation.

Series of six rats were irradiated with single doses from 3 to 30 Gy. Weight control was made at 2, 3, 5, 7 and 14 days after irradiation. Fig. 190 shows the reduction in weight and the spread in reaction. The dose of 15 Gy gives a maximum of reduction after about 7 days, when the weight starts to increase again. This dose of 15 Gy appears to be a crossroad between doses that turn to repair and doses that give continuing weight loss resulting in death or in a most critical condition between life and death.

Weight curves like these can be compared to the

presented SEM pictures of the condition of the mucosa and also to the summarizing of score-curves. After about 3 days, the reaction of the mucosa after 15 Gy seems to be maximal. The SEM picture after 7 days shows no change and still after 14 days the repair is not complete. In the score curve, a single dose of 15 Gy seems to represent a demarcation line just as in the weight-curves.

LD₅₀₍₃₀₎

As seen in Fig. 190 it could be assumed that the LD₅₀₍₃₀₎ in this particular partial abdominal irradiation would be in the region from 15 to 30 Gy. Single doses of 20 Gy, 22.5 Gy and 25 Gy were given to groups of 12 rats. The weight was followed during 30 days after irradiation. The results are presented in Fig. 191–Fig. 194. The LD₅₀₍₃₀₎ was estimated to be 22.5 Gy, Fig. 191.

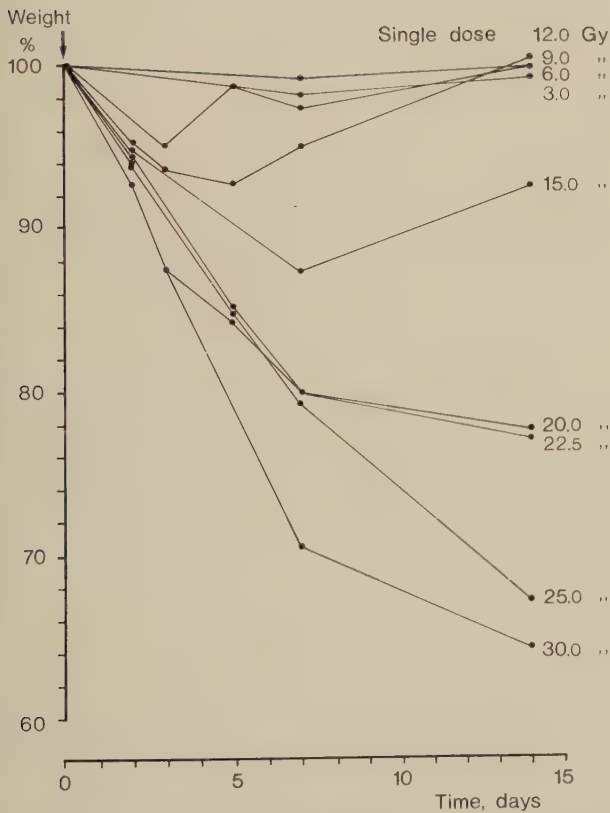


Fig. 190. Partial abdominal irradiation. Per cent change in weight after irradiation with single doses of 3–30 Gy. Standard deviation was less than 2% for curves < 15 Gy.

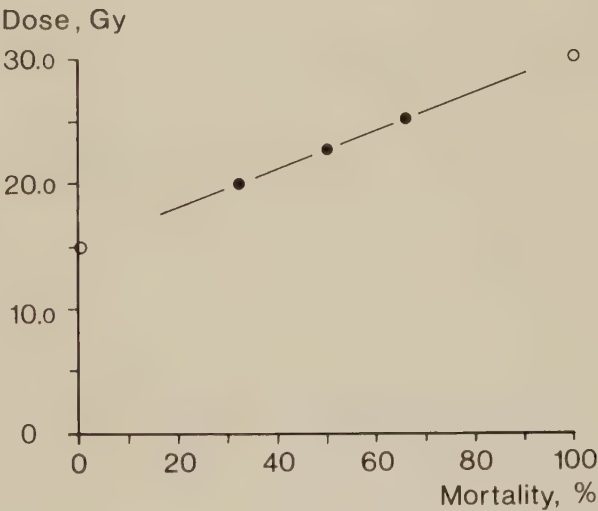


Fig. 191. Cumulative mortality within 30 days. The LD₅₀₍₃₀₎ is estimated to a single dose of 22.5 Gy.

This could be compared to the often reported intestinal death after 3–5 days or in later period after 5–7 days (*Quastler*, 1956). After 22.5 Gy as seen in Fig. 193, three animals decreased in weight rapidly until death within 7–10 days. After an increase,

some rats died in a second period after about 20 days.

In order to clarify this assumption, the curve in Fig. 193, with a given dose of 22.5 Gy, has been corrected by means of a computer: all weight curves

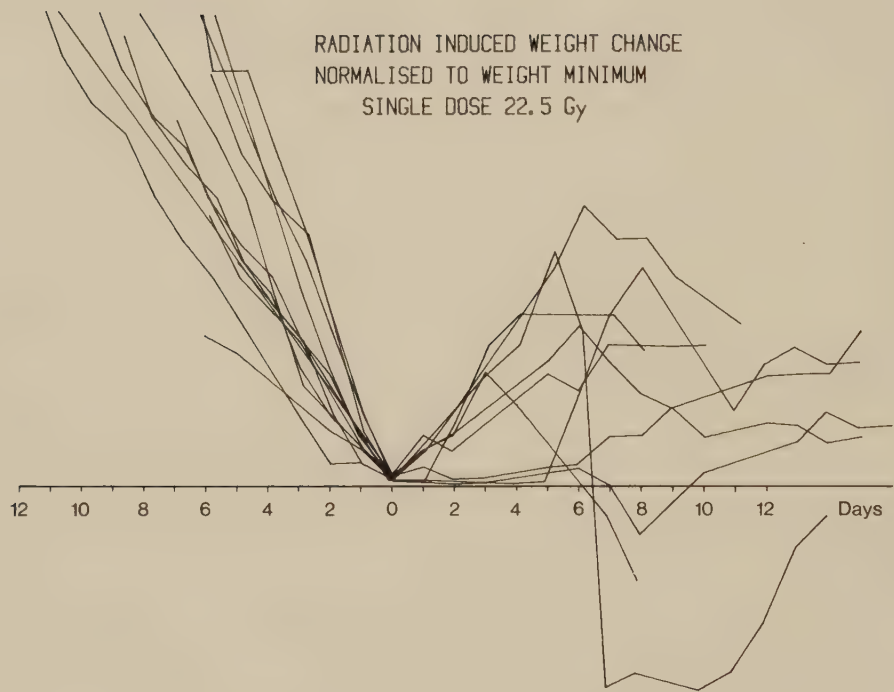


Fig. 192. Body weight. Single dose 22.5 Gy. All curves have been normalized to the first maximum decrease in order to visualize the second increase decrease phenomenon.

have been normalized to the first lowest point of weight loss (Fig. 192). At this time three rats had reached the point of no return. There seem to exist four types of phases in the weight curves, (Figs. 193 and 194). 1. decrease to a minimum where some

animals die, 2. increase in weight to a plateau, 3. the plateau between day 14 and 21 seems to be critical, 4. the animals who succeed in repair, gain in weight to the normal level from about day 21. Otherwise animals lose in weight again and die. The two

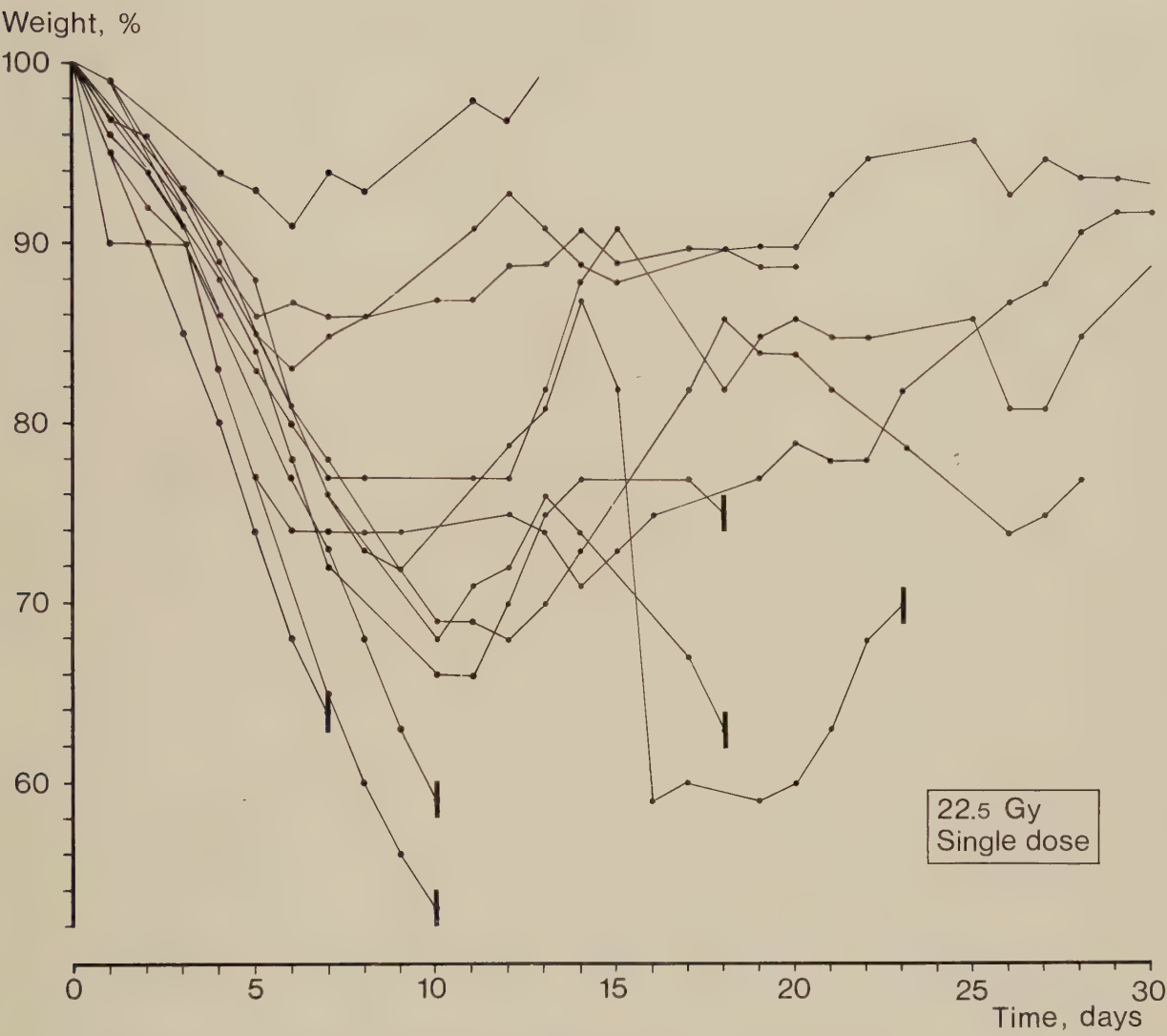


Fig. 193. Body weight. Single dose 22.5 Gy. Weight curve for each of 12 rats. This dose is the LD₅₀₍₃₀₎.

periods of death lie between 7–10 and 18–21 days.

The phase 3 in weight seems to be a sensitive phase. Explanation of this change in the curves is difficult. However, their principal appearance is supported by the findings of *Conard* (1956), who examined the weight of the small intestine, which is more or less a mirror of the actual weights

in this case. Another study of the body weight after abdominal irradiation by *Taketa* (1962), shows curves with a “hump” after the first decrease in weight. According to *Conard* (1956) these sequential changes reflect the activity in the crypts. This may be a result of the so called over-shooting.

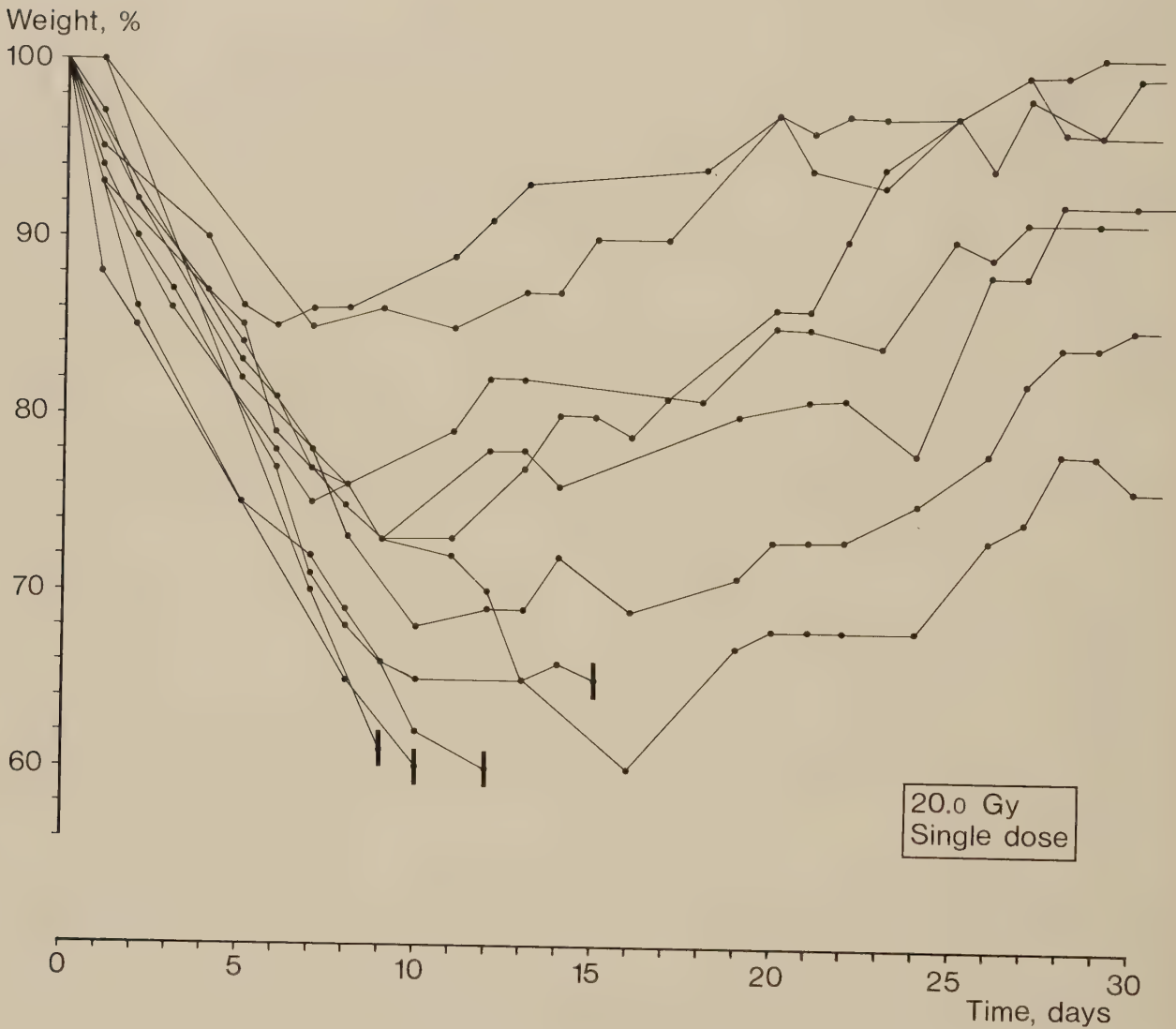


Fig. 194. Body weight. Single dose 20 Gy. Weight curve for each of 12 rats.

Discussion

By the SEM-technique it is possible to detect morphological changes down to $0.01\ \mu\text{m}$ on the mucosa due to a high resolution power, which allows a detailed study of the villi with epithelial cells, Goblet cells, microvilli and bacteria.

In spite of the fact that the SEM-technique is unable to demonstrate the crypts, the vascular bed, the lymphoid tissue and the stroma it is nevertheless possible – by following the course of events on the surface – to observe physiological changes in which both proliferation in the crypts, the vascularisation in the core and other parameters are involved. These changes are of such an importance that they cannot be neglected in judging the effect of irradiation. It has been considered convenient to divide the response to irradiation into different stages or groups depending on the dose given and the time after irradiation.

Within the short time of 60 minutes after doses between 15–30 Gy the following observations could be made: 1. swelling of the villi and the epithelial cells, 2. explosively emptying of the Goblet cells, 3. intense bacterial attack and 4. a breakthrough of lymphoid cells in Peyer's patches.

The swollen villi are retracted, have deep folds and epithelial cells with half-spherical surfaces. Slightly diverging microvilli may be a result of this changed cell surface not seen in the fasting animal. The bacteria seem to have penetrated violently. The cell surface has an enormous amount of mucinous granulae. Five minutes after 30 Gy, lymphoid cells pass through small openings in the membrane of the M-cells, starting with a narrowing of the penetrating cell and ending up with a spherical lymphoid cell with protrusions on the surface. The same reaction is seen after fractionated irradiation as e.g. $2\ \text{Gy} \times 5$, but to a minor degree. With increasing dose the Peyer's patch is declining, also seen at the fractionated dose of $5\ \text{Gy} \times 3$, Chapter VIII.

The explanation of this findings may be a strongly increased vascularisation with increased permeability (Jolles *et al.*, 1961) during the long irradiation time resulting in an activity much greater than in the non fasting animal. Normally the highest activity in the epithelial cells takes place in the upper third of the villus. The bacteria are attacking this part of the villi which does not contradict the assumed theory about bacteria discussed in chapter IV: that the reason should be a high production of precursors to the enteric surface also combined with a high activity in the microvilli of the disaccharidases. Becciolini (1970) found after irradiation a primary rise in the disaccharidases activity in the brush

border, followed by a decreased activity. A strong initiation to the Goblet cell calling for total emptying may also be a kind of stimulation. The penetration of lymphoid cells through the membranes of the M-cells is difficult to explain. In normal eating animals, see chapter IV, isolated breakthrough of lymphoid cells can be seen. May be a stimulation or a damage by the irradiation causes the M-cells to rupture. According to Owen (1979) lymphoid cells do normally not penetrate the M-cells into the intestinal lumen but the electron beam of the SEM ruptured the M-cell surface whenever focused on it. In this study, in fasting animals, no lymphoid cells penetrate the M-cells. Bellamy and Nielsen (1974) report neutrophile leucocytes and lymphocytes to be expelled between the epithelial cells of the villi to an extent of 10^7 cells/cm of guinea pig intestinal lumen. In this study no penetrating lymphoid cells have been seen penetrating the surface of the villi in normal or in irradiated animals. Ten minutes after an absorbed single dose of 25 Gy the reaction has decreased and after 20–30 minutes no bacteria are seen. Mucinous granulae are still seen on the cell surface and the villi are swollen. The bacteria do not return any more after high single doses – until repair. This reaction was reported after one single dose, 1000 rad (Porvaznik, 1979). When a repair is nearly complete the bacteria return again (see 9 Gy – 7 days). The absence of bacteria after 30 minutes and the existence of emptied Goblet cells may be signs either of exhaustion of the production capacity or a damage which can be compared to the damage of the production of steroid hormone receptors by ionizing irradiation (Alm *et al.*, 1979). A damage after 30 minutes cannot be explained by a sublethal damage to the crypt cells. These cells can hardly reach the functional compartment until after at least 24 hours.

Still 24–48 hours after single dose irradiation retraction of the villi is seen together with oedema. The surface seems not to be damaged morphologically the borderlines between the epithelial cells are demarcated and the folds clearly seen.

The damage to the crypts, not seen at all by SEM of the mucosal surface, is of importance in understanding the reactions that will come later on. The appearance during the first 48 hours after irradiation with almost normal villi of the mucosa surface stands in sharp contrast to the dramatic reaction going on at the same time in the crypts. A single dose of 1000 rad results in a loss of 80% of the crypt cells within four hours, Tsubouchi and Matsuzawa (1974). Hagemann *et al.* (1971) found that 50% of the crypts are destroyed after a single dose of 900 rad. Doses of 1400 R or above result in cell numbers reduced to zero and after 2400 R the

crypts are almost absent, *Devik* (1971). On the other hand sublethal damage is repaired very quickly. DNA-breaks are repaired again up to 50% within 15 minutes after a dose of 500 rad as shown by *Rydberg and Johansson* (1975). Different enzymes are active in this repair process, *Imondi et al.* (1969). A medium dose of 7 Gy gives a reduction in crypt cells reaching a maximum after 48 hours, *van der meer fieggen* (1973). At the same time cell proliferation starts and increases rapidly, giving a peak between 2 1/2 and 4 days, the so called overshooting-phenomenon. The normal number of cells is restored after 84 hours. After high single doses where no cells are left and the crypts disappear, crypts cannot be repaired for a long time, dependent on the size of the dose. After 1600 rad, for example, the increase in number of crypts does not start until 10 days after irradiation (*Cairnie*, 1971). When cell population in the crypts decrease, a not sufficient number of cells is delivered by migration to the villi, where the extrusion process expel hundreds of cells per hour. *Lajtha and Oliver* (1962) report a reduced migration rate of the epithelial cells. *Leshner* (1967) finds no stop in migration of cells from the crypts after irradiation. Normally about 100 epithelial cells are extruded from each villus per hour, according to the finding on the artifact pictures, (chapter V), where about fifty cells surrounds the upper part of a villus. The labelling technique shows a migration rate of two cell widths per hour (*Leshner and Bauman*, 1969). As is also seen from the artifact pictures, Fig. 45 every villus is surrounded by approximately 10 crypts, each probably delivering cells to two villi. The result may be that every crypt has to deliver 20 new cells per hour. The normal production of new cells in each crypt is measured to be about 13 cells per hour, *Hagemann et al.* (1970). As a result of the reduced number of crypt cells an intensive proliferation is started. The whole crypt is involved in the DNA synthesis and the mitosis is enormous. The turn-over time is reduced, dependent on the dose size, to a minimum of 7–8 hours (*Leshner and Bauman*, 1969) against the normal of 36–48 hours.

Normally a kind of control mechanism keep the proliferative compartment constant regarding production of new cells. As a result of irradiation an increased proliferation may guarantee that the period of stop normally found in production shall be compensated for, by a delivering of new cells to the villus. It has been stated that a feedback mechanism between the villus and the crypt regulates the effect and repair of irradiation (*Rijke et al.* 1974). A signal from the villus report the lack of new cells to the crypts, which then react by an overproduction of cells. This hypothesis assumes that a reduction of

60–70% of the villus' height will trigger an expansion of the proliferation compartment in the crypt.

Based pm these SEM-studies of the surface of the mucosa after single dose irradiation it seems to be possible to suggest another hypothesis depending on the appearance of the villus after different doses. Thus five levels of reaction of the villi can be isolated..

I. Low single doses as 2 and 3 Gy don't show any morphological changes of the villi. The only reaction is a kind of stimulation, mentioned existing for a few days.

II. 6 Gy makes the villus change in configuration into a pyramidal shape but there is almost no extrusion of cells on the tops and the bacteria are still penetrating the epithelial cells to a moderate degree around the tops. No reduction in height can be observed. The maximum reaction, as described, comes after 3 days. After 5–7 days a normal villus is restored. In the crypt this dose results in a reduction of the number of cells (*Leshner and Leshner* 1974), but the cell number increase again after two days (*Devik* 1971). The change in villus configuration to pyramidal form, Fig. 101, may be a state of preparation to increased extrusion of cells and reduction in height if the migration of new cells up to the villi should be inhibited too much. Apparently a feedback mechanism by communication from the crypt to the villus may exist. The lag period before new cells have replaced those lost by irradiation seems to be short enough for the villus to avoid any reduction in height. As seen in Fig. 148, 48 hours after such a high single dose as 25 Gy, the start of an increased extrusion is not seen until this moment at 48 hours. The lag period for the villus to react morphologically may be estimated to about 48 hours. The normal turn-over time for the small intestine is 36–48 hours. The dose for start of reduction in height of the villi seems to be 6 Gy.

III. At 9 Gy the reaction has increased. The pyramidal form can be seen but also groups of cells in extrusion process on the top of each pyramide (Fig. 108, after 72 hours). The folds are not accentuated which can be an extra sign of retraction because of extrusion of a lot of cells. Thus the damage to the crypt must be large enough to make the villi react by reduction. According to *Devik* (1971) the increase in crypt cells will not start until after three days after a single dose of 8–9 Gy. The lag period has passed 48 hours, which seems to be a critical point of time for the villi. As can be seen in Fig. 108 there are no bacteria any longer, indicating that a damage has stopped the function of the epithelial cells or the microvilli, through which the

products pass to the surface. A third possibility is a damage in the crypt in such a way that cells which migrate to the upper part of the villi are insufficient in differentiation or function.

IV. As seen in the weight curve, Fig. 190, and in the score curves, Fig. 186–188, 15 Gy is a kind of crossroad between life and death for the animals. A maximum damage is seen at day 3 after irradiation. This reaction is then going on with only minor change for about one week before the process of repair restore the normal villi and the weight is regained. The height is estimated to be reduced to about 50%. A massive extrusion is seen (Fig. 116) on some villi but at the same time others have a plain surface where the extrusion obviously is stopped. The feed-back mechanism here may result in a steady state from this moment. The extrusion seems to continue on some villi at 5 and 7 days, and the LM control shows partly destroyed crypts and columnar cells replaced by squamous cells on the surface. No denudation or ulceration can be seen and no bacteria. Thus the mucosa is intact and the framework, the core, has not disappeared. Repair can be observed after 14 days and is going on after 21 days. As reported by (Cairnie, 1971) crypts do not reach control levels until after 30 days after 1600 rad.

V. In this kind of reaction level still higher doses result in serious damage, which, however, not necessarily do result in death. 22.5 Gy and 25 Gy start a massive extrusion from the tops of pyramidal villi (Fig. 148) that does not stop until all villi have disappeared and the mucosa is a plain surface. The maximum effect of irradiation is at the end more or less advanced ulceration. The break-down from normal height to plain surface takes only about 48 hours and starts 48 hours after irradiation. There is no stop in the extrusion and small islands of ulceration appear. Thin, large omegacells constitute the remaining mucosal surface. The dose of 22.5 Gy has been found to be the $LD_{50(30)}$ and as seen from the weight curves, Fig. 193, some animals will die after 7–10 days. Other animals, who survive, decrease in weight but regain weight to a level, which is stationary until about day 21–25. This period seems to be critical. Some animals do not survive and die after 18–21 days. The impression from SEM is that if the ulceration is less than 50% the animal has a chance. After 21 days new villi grow up irregularly from a new intact surface and this condition could be compared to the weight increase that cannot start until a restored mucosa and functioning villi are built up. During the critical period disturbances in leakage of proteins and loss of electrolytes may result in death at any time. In

spite of the damage no bacteria can be seen during this period. Still toxins may enter through the open surface and kill the animals.

These five levels of reactions are a summary of the findings. Before a break-down through extrusion of cells there exists a lag period. *Patt and Quastler* (1963) have shown that the denudation is postponed. As seen in this study the lag period is about 48 hours, even after high single doses. The feed-back mechanism is presented by *van der meer-fieggan* (1973) and *Rijke et al.* (1974) as a signal from the villus to the crypt when not sufficient cells are migrating to the villi. However, an information in the opposite direction cannot be excluded. When no new cells are produced in the crypt the villus simply has to react by preparing a reduction by taking a pyramidal form and later on start a large extrusion process. If the damage is small the lag period will be enough for the crypt to produce new cells to the villus in time. But if the damage is large, the villus have to break down by a massive extrusion to a total disappearance. The reason may be a lack of new cells but also a disappearance of the core or the framework. With maximal proliferation and the crypts extended by hundreds of cells the animal may still die because of an ulcerated mucosa. If the building of a new framework goes on the migration of crypt cells will start again and new villi grow up irregularly. The large number of Goblet cells in the crypts seen after irradiation is also present on the new growing villi.

A few statements can be summarized from the SEM pictures of the single dose irradiation effects.

- A. the function as measured by the attack by bacteria is damaged dependant on dose and time. After high doses, 22.5–25 Gy, bacteria disappear after only 30 minutes and will not return until after full repair. Lower doses up to 6 Gy result in an increased attack by bacteria but at doses higher than 6 Gy they disappear together with the starting of the morphological damage of the villus after 48 hours. At doses of 6–12 Gy bacteria attack the restored villi again after one week.
- B. the lag period before any morphological damage seems to be 48 hours.
- C. the extrusion process, prepared by a pyramidal form of the villi, does not start until after the lag period of 48 hours.
- D. total disappearance of the villi together with different degrees of ulceration is seen after doses higher than 15 Gy after 5–7 days.
- E. death in this partial abdominal irradiation occur in an early period between 7–10 days. There exists a critical period between 14 and 21 days

during which growth of new villi is seen but in which period some animals may die in a late period, 18–21 days after irradiation. The weight curves confirm these findings.

The critical single dose has been shown by *Devik*

(1971) to be 1200 R in whole body irradiation. *Withers and Elkind* (1968) found regeneration of the crypt cells up to a single dose of 2000 R. In this study (partial abdominal irradiation) the critical single dose seems to be 15–20 Gy.

Chapter VIII

Fractionated irradiation

Introduction

In an effort to simulate the fractionation schedules used clinically in radiotherapy, the conventional method of five fractions per week was studied with a total time of three weeks and a total dose of 30 Gy. A fraction of 2 Gy was given every day from Monday to Friday. In order to control if marginal increase or decrease in daily dose resulted in any different reactions, a study was performed with 1.8 Gy/day and 2.2 Gy/day.

Other schedules were studied, such as three fractions per week given on Monday, Wednesday and Friday as 4 Gy, 3 Gy and 3 Gy, respectively and as two fractions per week with 5 Gy on Monday and Friday. Total time and dose were kept at 3 weeks and 30 Gy in the schedules presented.

Further on, small fractions of 1 Gy in a so-called super-fractionation scheme were given 3 times a day (at 8 o'clock a.m., 4 o'clock p.m. and at mid-night) five days a week up to 3 weeks with a total dose of 45 Gy. This schedule was doubled and metronidazole was given around the clock in the water during the overall time of 3 weeks in a dose of 24 mg/day/rat.

In all schedules, 2 rats were sacrificed each day, 24 hours after given dose. On Mondays, 72 hours after irradiation, specimens were also taken for SEM-study. In the schedule of 1 F/week, rats were sacrificed 72 hours after each of the 3 doses in order to see the maximum effect.

When the schedules of fractionated irradiation were completed after 3 weeks, specimens were taken at day 3, 7 and in some series up to 28 days after irradiation.

In Chapter VII, the reaction of different single doses at different times were presented in a score system where the conclusion of all doses resulted in effect-curves. The whole scale of the injuries could now be used as a base for judging the reaction following fractionated irradiation.

Until now no SEM-study of the small intestine, including high magnification, has been done of partial abdominal fractionated irradiation, and it was therefore considered necessary to be able to use the

effects of the single dose irradiation as a comparison. The single dose effects in SEM on the other hand can be compared to the vast experience from other kinds of technique, as LM and TEM.

In order to study if metronidazole could effect the attack by bacteria, characterized as anaerobe in type, it was desirable to include experiment with this sensitizer, as well as a test with mannose, with regard to the findings described in Chapter IV in non-fasting animals. The body weight of the animals was recorded daily. Table 2 shows the survey of the given doses and the fractionation schedule.

Table 2. Fractionation schedules showing fraction size and number of fractions.

Day	Number of fractions						
	1/w	2/w	3/w	5/w			15/w
1	9.0	5.0	4.0	1.8	2.0	2.2	1.0×3
2				1.8	2.0	2.2	1.0×3
3			3.0	1.8	2.0	2.2	1.0×3
4		5.0		1.8	2.0	2.2	1.0×3
5			3.0	1.8	2.0	2.2	1.0×3
6							
7							
8	9.0	5.0	4.0	1.8	2.0	2.2	1.0×3
9				1.8	2.0	2.2	1.0×3
10			3.0	1.8	2.0	2.2	1.0×3
11		5.0		1.8	2.0	2.2	1.0×3
12			3.0	1.8	2.0	2.2	1.0×3
13							
14							
15	9.0	5.0	4.0	1.8	2.0	2.2	1.0×3
16				1.8	2.0	2.2	1.0×3
17			3.0	1.8	2.0	2.2	1.0×3
18		5.0		1.8	2.0	2.2	1.0×3
19			3.0	1.8	2.0	2.2	1.0×3
20							
21							

Two rats were sacrificed and examined every day after the first day of irradiation in all regimes.

Fract. irr. 5 F/week



Fig. 195. Fract. irr. 3 × 2 Gy. Pyramidal villi, swollen. Increased extrusion. Bacteria at the tops. 280 ×.

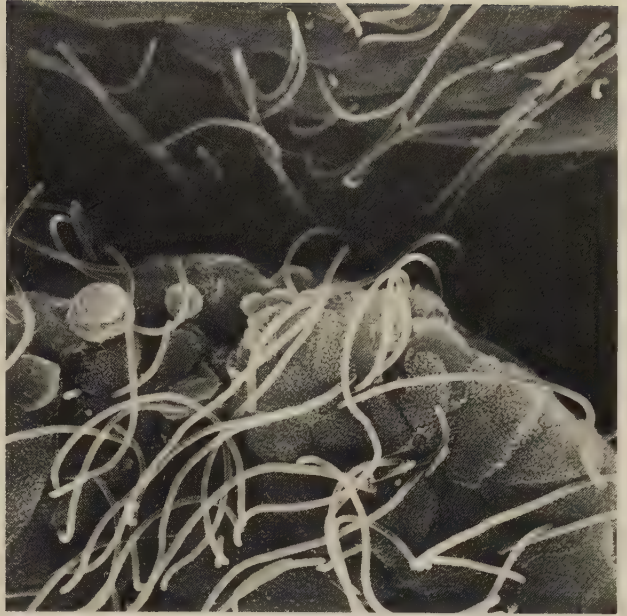


Fig. 196. Fract. irr. 3 × 2 Gy. Top of a villus with bacteria and loosening cells. 1100 ×.

Schedule: 5 F/week. (2 Gy × 5/week)

This conventional fractionated irradiation was given Monday to Friday for three weeks with a fraction of 2 Gy every morning. 2 rats were removed every 24 hours for SEM preparation and LM-preparation. After total dose, 30 Gy, the reaction of the ileum was followed up to 28 days later. The schedule was repeated. Using the same fractionation regime, two other series were performed with a fraction of 1.8 Gy and 2.2 Gy.

As seen in chapter VII, 2 Gy in single dose results, as studied by SEM, in no detectable morphological reaction, but gives a state of high activity shown as increased attack by bacteria, oedema, Goblet cell secretion and lymphoid cell penetration.

The comparison of the average reaction versus dose after irradiation is presented in Fig. 340. At a dose of 10 Gy, a score between 0.5–1.0 is attained and is nearly unchanged during the following two weeks. This finding is parallel to certain other fractionation schedules and will be described below in greater detail, since the regime simulates general clinical treatment.

3 × 2 Gy

Villi show a pyramidal form with increased extrusion at the top and an increased number of bacteria

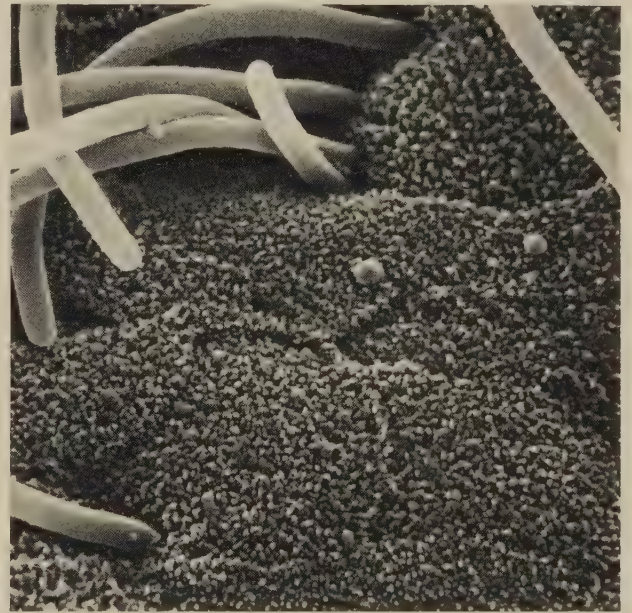


Fig. 197. Fract. irr. 3 × 2 Gy. Diverging microvilli on the epithelial cells. Bacteria. 5200 ×.

penetrating epithelial cells with microvilli diverging and with various diameter of the tips, Figs. 195–197. This change in the cells is especially pronounced at the upper parts of the villi.



Fig. 198. *Fract. irr.* 4×2 Gy. Slender pyramidal-formed villi. Swollen tops. Few bacteria. $400 \times$.



4×2 Gy

At the end of the first week and with 8 Gy given, the villi stand erect and regular and have normal height. They are of pyramidal form, swollen at the tops, where only a moderate number of bacteria penetrate. The folds are not changed. In high magnification mucinous granulae are spread everywhere on the surface, Fig. 199. The microvilli on the side wall cells are tightly packed.

Fig. 199. *Fract. irr.* 4×2 Gy. Side of a villus. Three bacteria penetrate at the same point. Lots of mucinous granulae on normal microvilli surface. $10,000 \times$.



Fig. 200. Fract. irr. 5×2 Gy. Narrow pyramidal villi. Bacteria only at the tops. 400 $\times$.

5×2 Gy

24 hours after five fractions the villi have about the same appearance as after 4 fractions. They are narrow and have more bacteria around the highest part, attacking the epithelial cells that are beginning

the extrusion process. The folds have not changed. The Goblet cells explosively empty their granulae as seen on the cell surface and on LM (Fig. 203 and 208). In comparison with 3×2 Gy the cells on the top of the villus are more swollen with some cells almost spherical (Fig. 201 and 203). On the side wall



Fig. 201. Fract. irr. 5×2 Gy. Top of a villus. Spherical cells. Attack by bacteria. Diverging microvilli. $10,000 \times$.

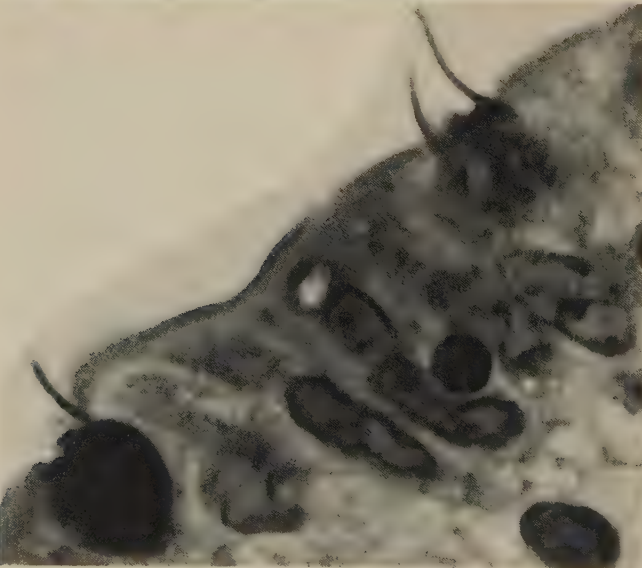


Fig. 202. Fract. irr. 5×2 Gy. LM from the villus top. Bacteria.

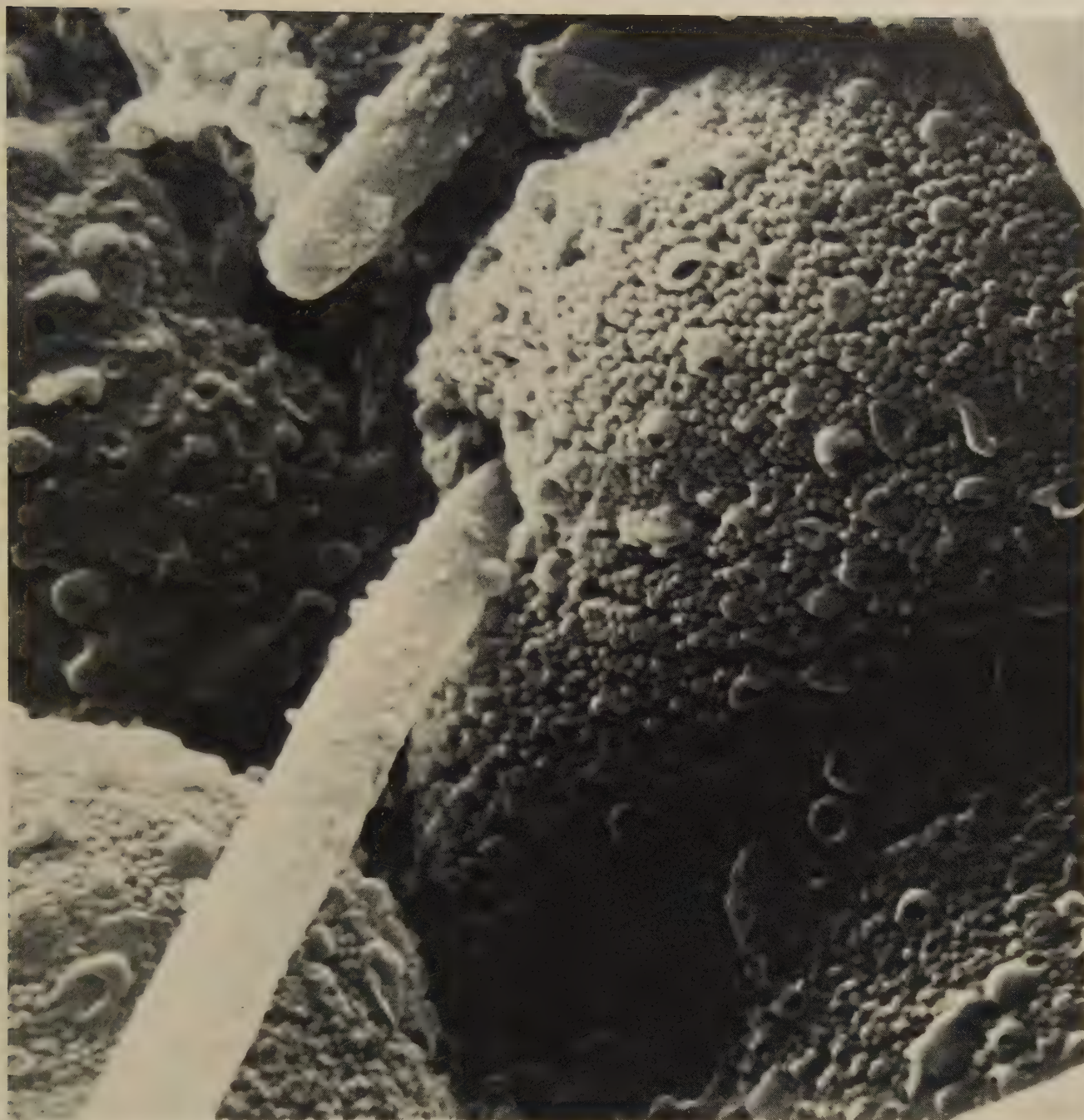


Fig. 203. Fract. irr. 5×2 Gy. One spherical cell penetrated by bacterium. Mucinous granulae on the surface. 20,000 $\times$.

not much reaction can be seen. The microvilli are more diverging on some cells (Fig. 209). Bacteria penetrate at the vertex of the swollen cells but also at the borderlines. This pattern of penetration is difficult to explain. Perhaps some part of the cell is more attractive at the place where two or more bacteria attack (Fig. 201). The head of the bacterium

penetrating a cell seems to be spear-like (Fig. 204 and Fig. 206). The process with lymphoid cells coming through the membrane of the M-cells is seen in a sequence in Fig. 210–213. Again the state of increased activity may be expressed by this cell leakage.

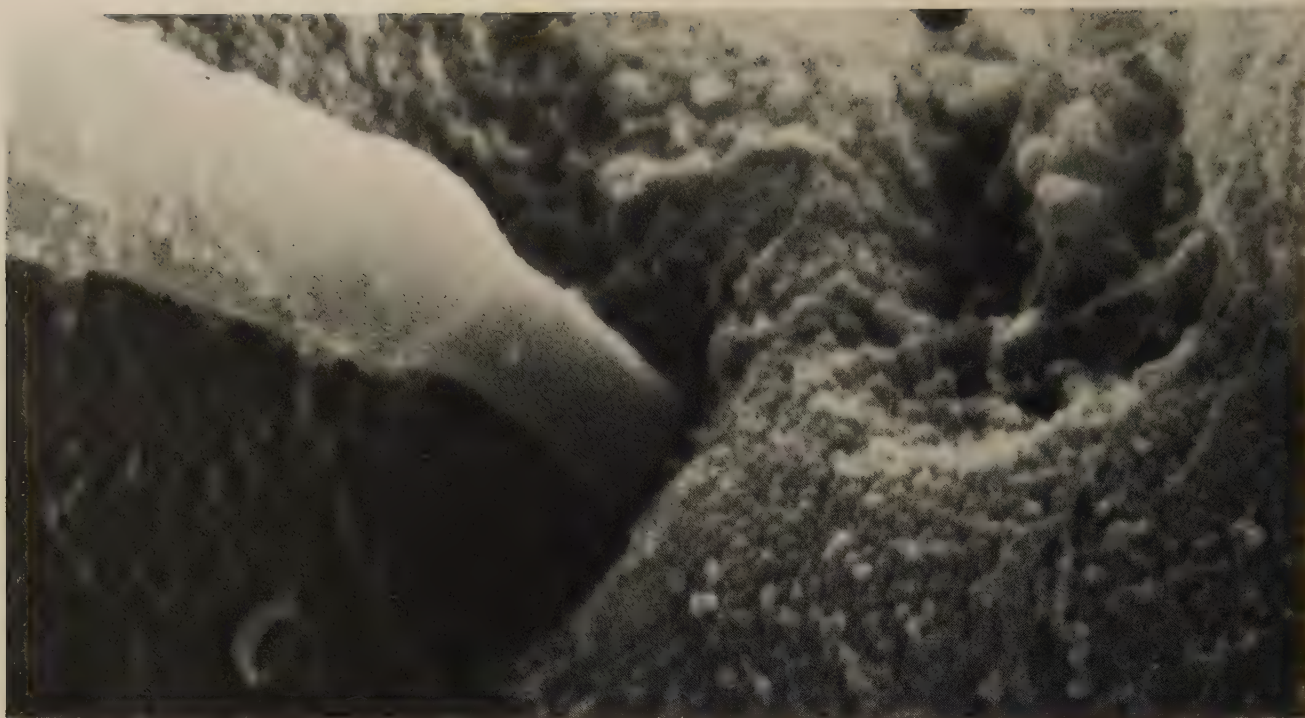


Fig. 204. *Fract. irr. 5 × 2 Gy.* Bacteria narrow at the penetrating end. Closed Goblet cell at the right. 20,000 ×.

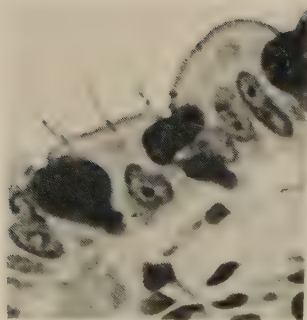


Fig. 205. *Fract. irr. 5 × 2 Gy.* LM showing the same thing as Fig. 204 and Fig. 206.

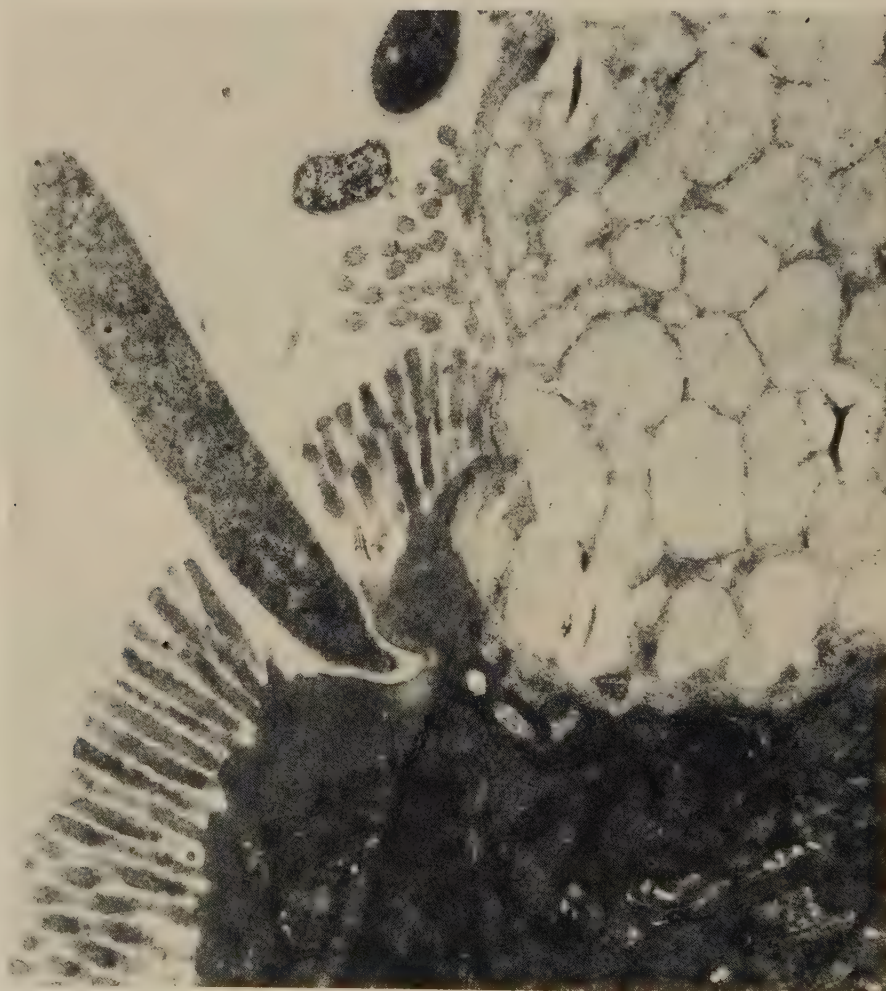


Fig. 206. *Fract. irr. 5 × 2 Gy.* TEM picture of penetrating bacteria as seen in Fig. 204. Unemptied Goblet cell. 29,600 ×.



Fig. 207. Fract. irr. 5×2 Gy. Side of a villus. Almost normal cells, of polygonal form. $2100 \times$.

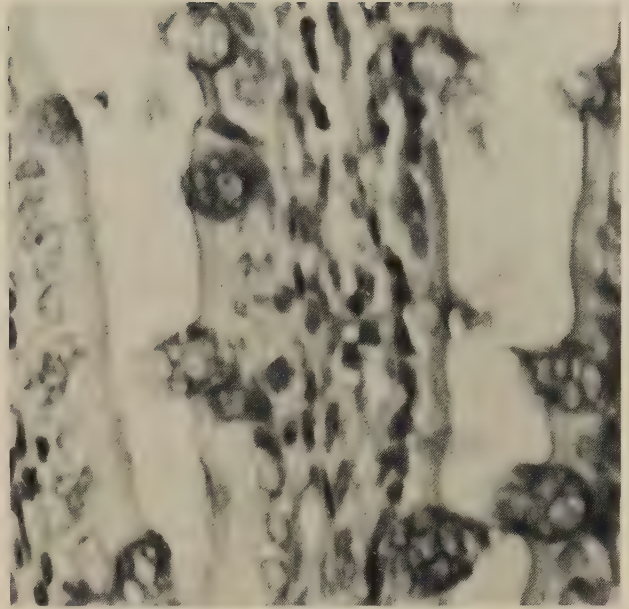


Fig. 208. Fract. irr. 5×2 Gy. LM showing emptying Goblet cells on the side of the villi.

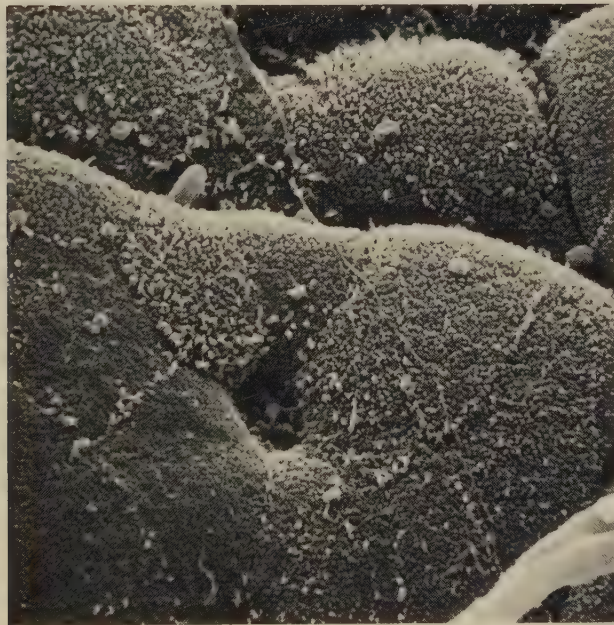


Fig. 209. Fract. irr. 5×2 Gy. Part of Fig. 207. Some cells have diverging microvilli. $4700 \times$.



Fig. 210. Fract. irr. 5×2 Gy. Peyer's patch. Lymphoid cell passing through the membrane of a M cell. 4700 $\times$.



Fig. 211. Fract. irr. 5×2 Gy. More cells penetrating. 4700 $\times$.

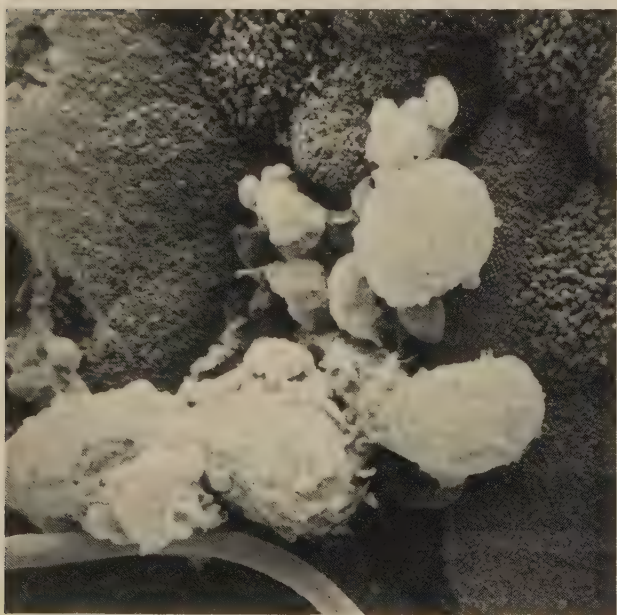


Fig. 212. Fract. irr. 5×2 Gy. Intensive leakage of lymphoid cells of different shapes. 4700 $\times$.



Fig. 213. Fract. irr. 5×2 Gy. No more cells. Note the narrow opening or hole. 4700 $\times$.

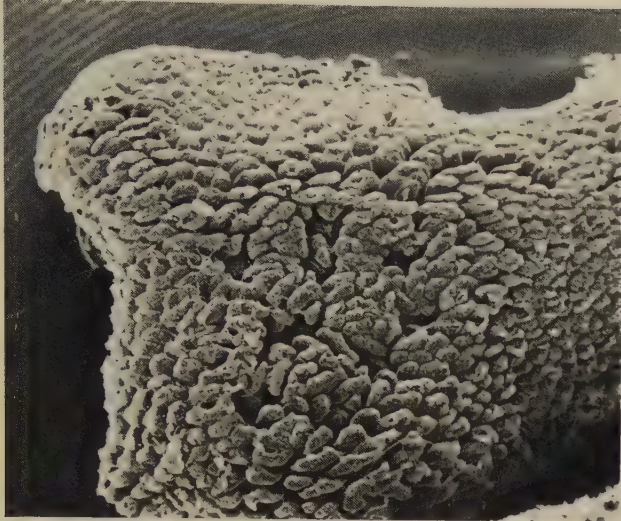


Fig. 214. *Fract. irr. 7×2 Gy*. Low magnification. Peyer's patch in the center, hardly visible. 20×.

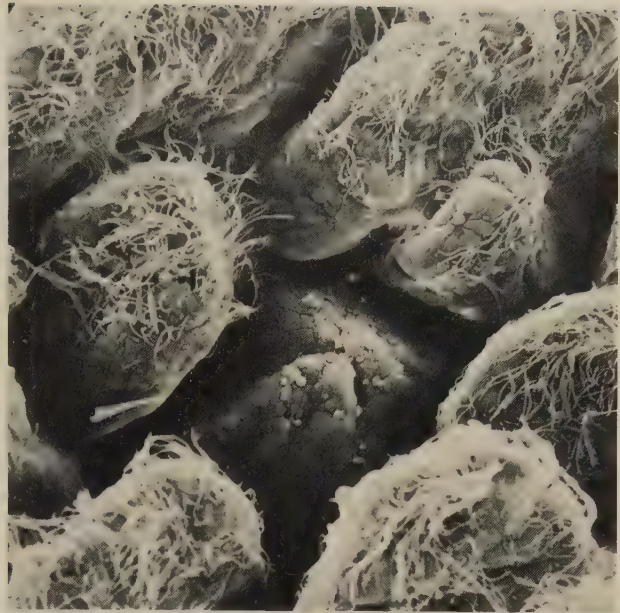


Fig. 215. *Fract. irr. 7×2 Gy*. Villi with bacteria surrounding shrinking nodule of Peyer's patch with no leakage of cells and no bacteria. 200×.

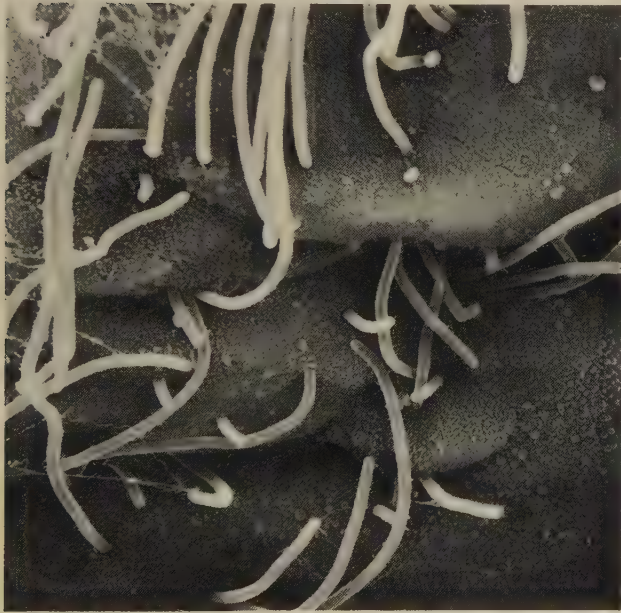


Fig. 216. *Fract. irr. 7×2 Gy*. Attacking bacteria on the side of a villus. Normal epithelial cells. 2000×.

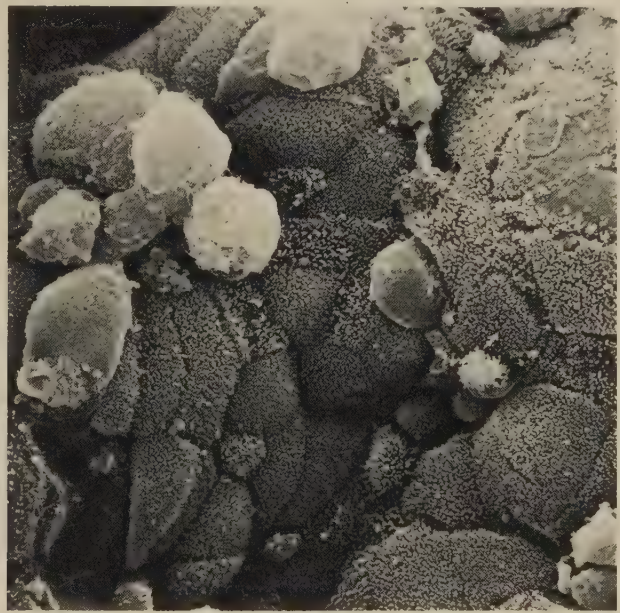


Fig. 217. *Fract. irr. 7×2 Gy*. Peyer's patch. Epithelial cells with diverging microvilli. No M-cells. 2000×.

7×2 Gy

The same reaction is still recorded confirming a kind of steady state. Peyer's patches are not to be found in every specimen. Fig. 214 and Fig. 215 show rudimentary nodules hidden between the villi. The

lymphoid tissue must be damaged. There are no lymphoid cells coming through and no bacteria are found in the center or on the tops of the villi (Fig. 215).

The differences between microvilli can only be



Fig. 218. Fract. irr. 7×2 Gy. Top of a villus. Various kinds of microvilli. Bacteria. Probably minor irradiation reaction. $10,000 \times$.

recorded at high magnification on SEM (Fig. 218 and Fig. 219).

It is difficult to explain the deviation from the normally even and closely packed carpet of micro-

villi, since basic functions appear roughly normal. Mucinous granulae can be seen and bacteria are penetrating, both signs of maintained function of the villi.

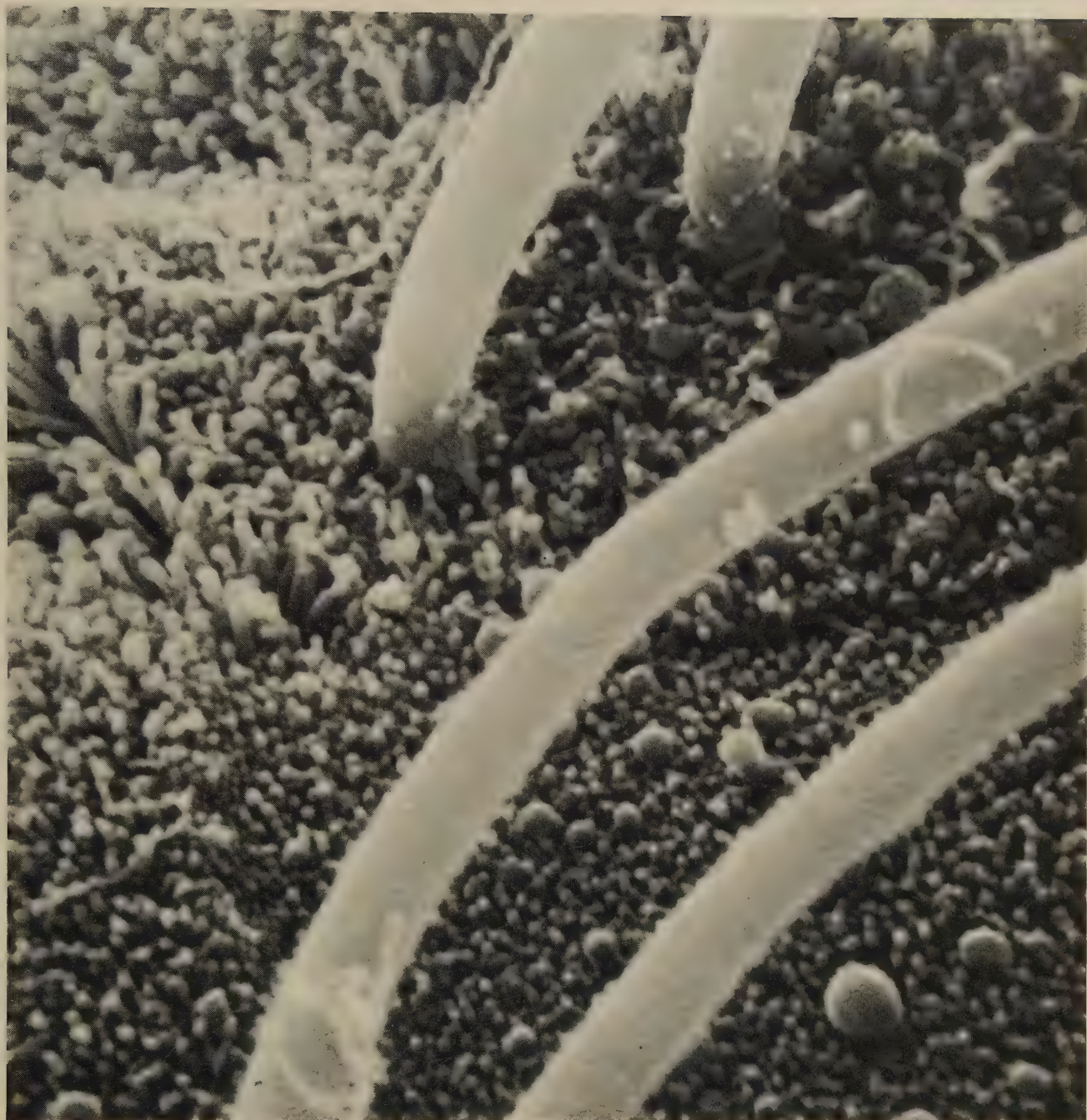


Fig. 219. Fract. irr. 7×2 Gy. Enlargement of Fig. 218. Short or long, swollen or non-swollen microvilli. 20,000 $\times$.

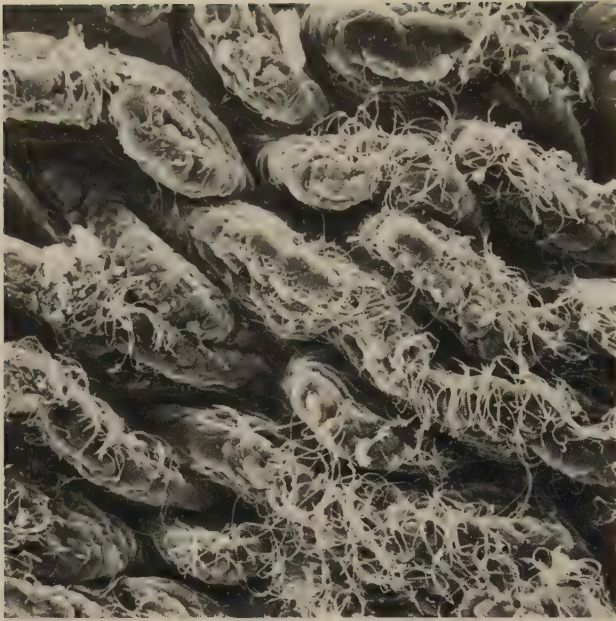


Fig. 220. Fract. irr. 8×2 Gy. Pyramidal form of the villi. A lot of bacteria. 200 $\times$.

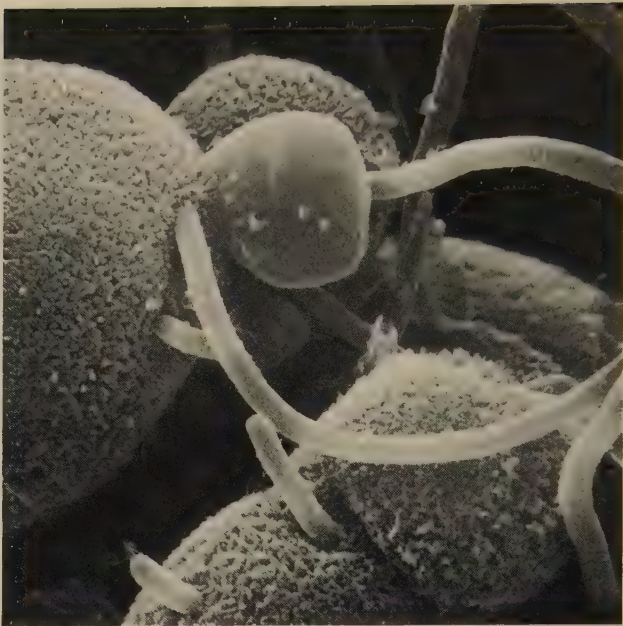


Fig. 221. Fract. irr. 8×2 Gy. Top of a villus. Spherical epithelial cells, Bacteria. 5000 $\times$.

8×2 Gy

There is an increased number of bacteria around the tops of the pyramidal villi (Fig. 220). No other new reaction can be seen.



Fig. 222. Fract. irr. 8×2 Gy. Many bacteria attacking the same, loosening cell. 4000 $\times$.

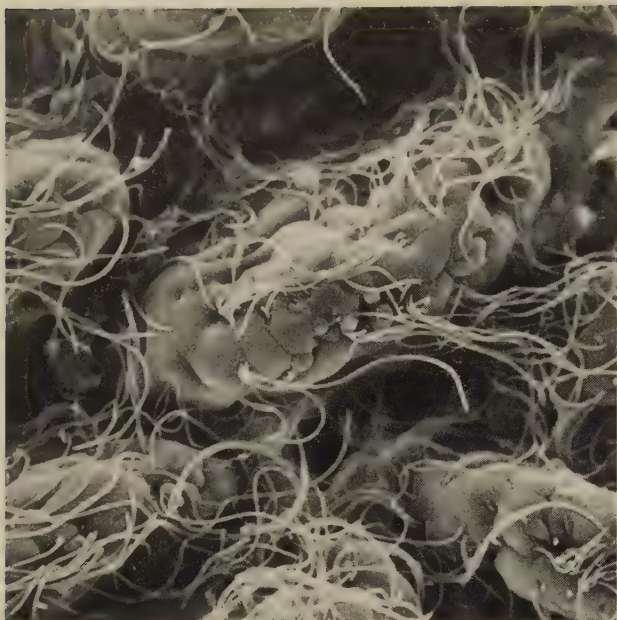


Fig. 223. *Fract. irr. 10 × 2 Gy.* Bacteria on the tops. Increased extrusion. 500 ×.

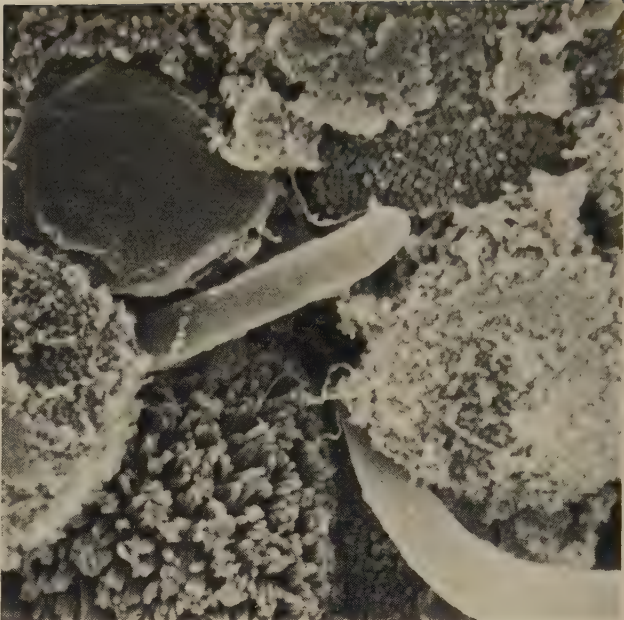


Fig. 224. *Fract. irr. 10 × 2 Gy.* Cells on the villus top. Spherical cells. Diverging microvilli. 10,000 ×.

10 × 2 Gy

After 2 weeks, 10 fractions and 20 Gy, there is still a kind of steady state with no change as seen at different magnifications. The attack by bacteria, as shown in LM in Fig. 225, is concentrated to a few cells. In spite of repeated fractions of 2 Gy the villus

does not show any increased extrusion or further retraction. The number of new cells migrating up from the crypts must be enough in quantity and qualitatively the villi are functioning. The weight curve shows a small reduction, and the anaesthesia itself is responsible for part of it.

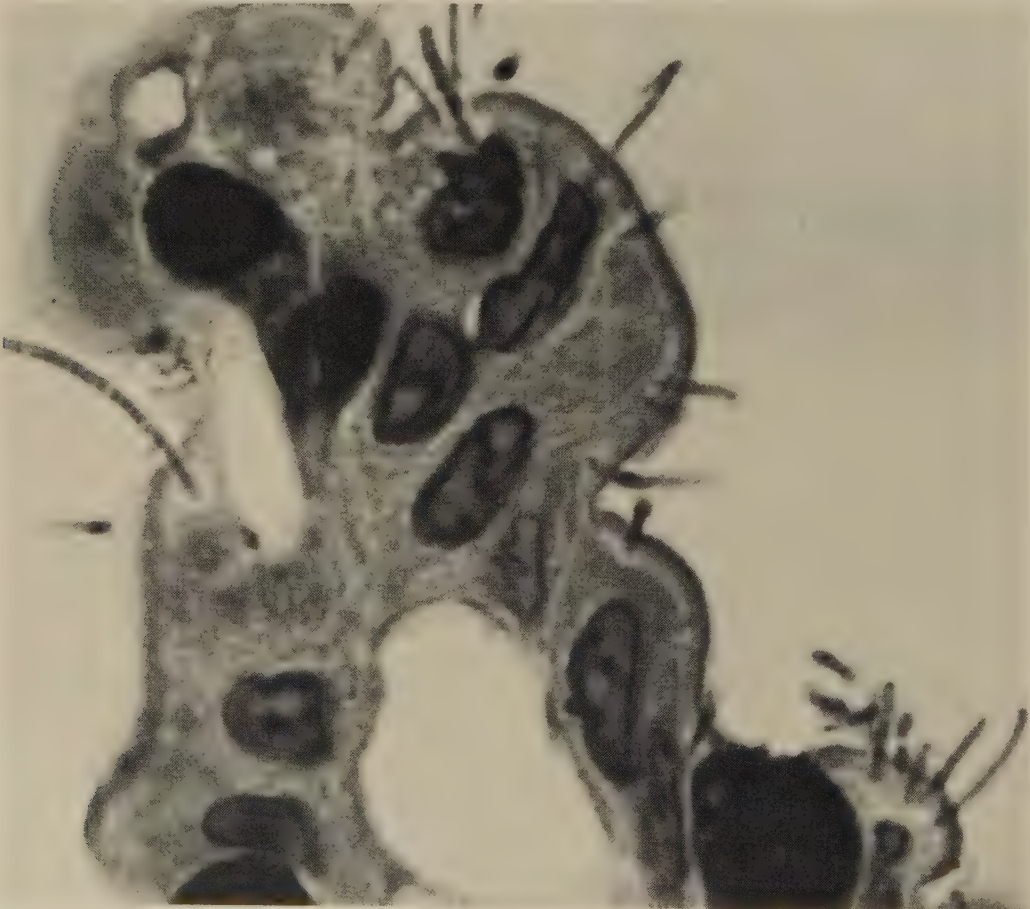


Fig. 225. *Fract. irr. 10 × 2 Gy.* LM of top of a villus. Bacteria concentrated on a few cells.



Fig. 226. Fract. irr. 11 × 2 Gy. Part of villus surface with diverging microvilli on all cells. 2000 ×.



Fig. 227. Fract. irr. 12 × 2 Gy. The same reaction as in Fig. 226. 2000 ×.

11, 12 and 12 × 2 Gy

Still the same reaction. The pictures show the diverging microvilli on every epithelial cell as after 7 × 2 Gy.



Fig. 228. Fract. irr. 13 × 2 Gy. High magnification of short diverging microvilli on epithelial cells. 4700 ×.



Fig. 229. *Fract. irr. 15 × 2 Gy – 3 days*. Swollen pyramidal villi. Some bacteria. 190 ×.

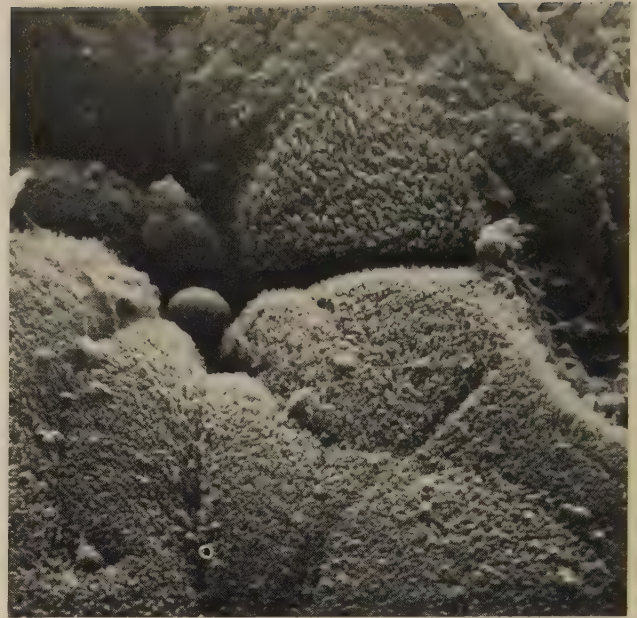


Fig. 230. *Fract. irr. 15 × 2 Gy – 3 days*. Diverging, probably short, microvilli. No swelling of the cells. 4700 ×.

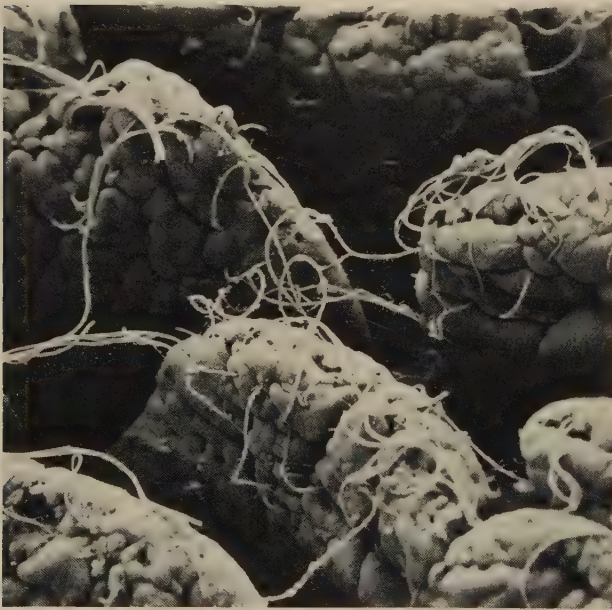


Fig. 231. *Fract. irr. 15 × 2 Gy – 7 days*. Villi with long ridges. Swelling seen as deep folds. Bacteria. 360 ×.



Fig. 232. *Fract. irr. 15 × 2 Gy – 7 days*. Tightly packed microvilli. 9000 ×.

15 × 2 Gy

24 hours after completed schedule of 30 Gy in total dose and 72 hours later there are nearly the same signs of reaction. Villi have a narrow or pyramidal top. Bacteria are attacking the tops to the same degree. The epithelial cells are perhaps not swollen to the same degree as earlier. The microvilli still have diverging tips. In conclusion a steady state was found during the irradiation, but already 3 days later a more normal situation was established.

15 × 2 Gy – 7 days later

Swollen villi. Bacteria to a normal extent. Diverging microvilli.

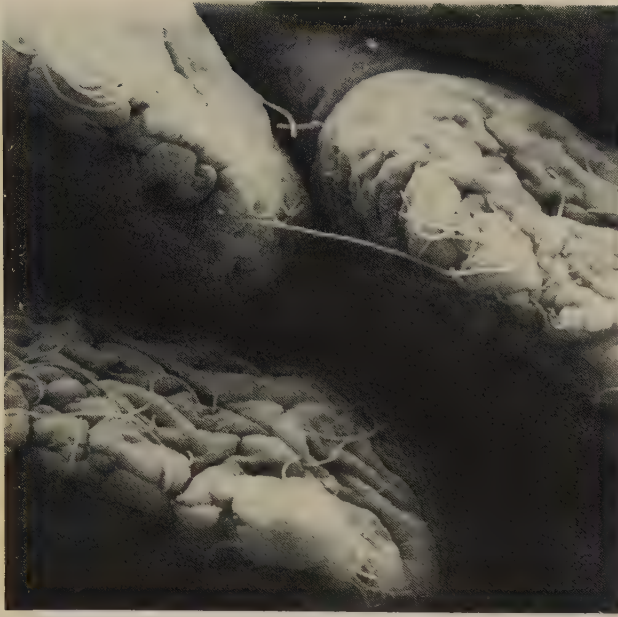


Fig. 233. Fract. irr. 15 × 2 Gy – 14 days. Swollen but almost normal villi. No increased extrusion. Moderate degree of bacteria. 470 ×.



Fig. 234. Fract. irr. 15 × 2 Gy – 14 days. Peyer's patch. Normal surface. No penetration of cells. Bacteria in the central area again. 470 ×.



Fig. 235. Fract. irr. 15 × 2 Gy – 28 days. Normal, high villi with long ridges. The bottom between them can be seen. 190 ×.

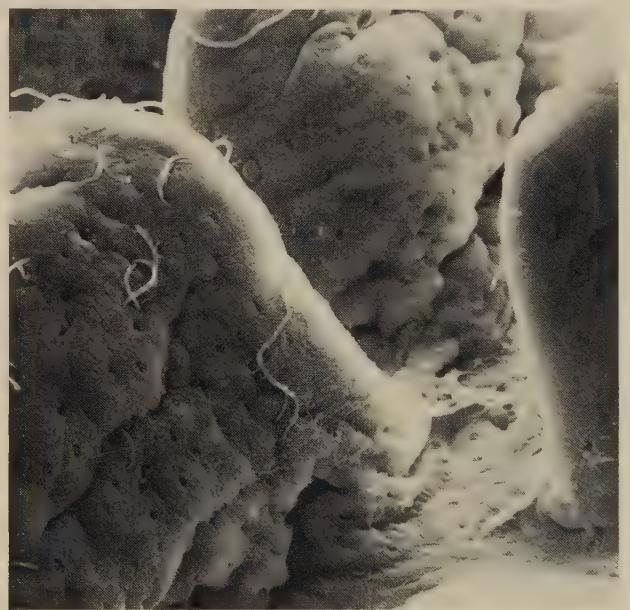


Fig. 236. Fract. irr. 15 × 2 gy – 28 days. All over the side of the villi there are closed Goblet cells. Normal picture. 470 ×.

15 × 2 Gy – 14 days later

Almost no difference in appearance from a normal condition. Observe the normal Peyer's patch with bacteria attacking in the central area (Fig. 234).

15 × 2 Gy – 28 days later

High, slender villi. Many Goblet cells are seen on the side of each villus. During the irradiation most of the Goblet cells have emptied their mucinous granulae and may not be working any more. Therefore the openings are small and nearly closed. The villi have a long ridge at the top. The epithelial cells are no longer spherical and the microvilli are normal. The regeneration is completed within 14–21 days after irradiation. The weight reduction has been regained during the same time.

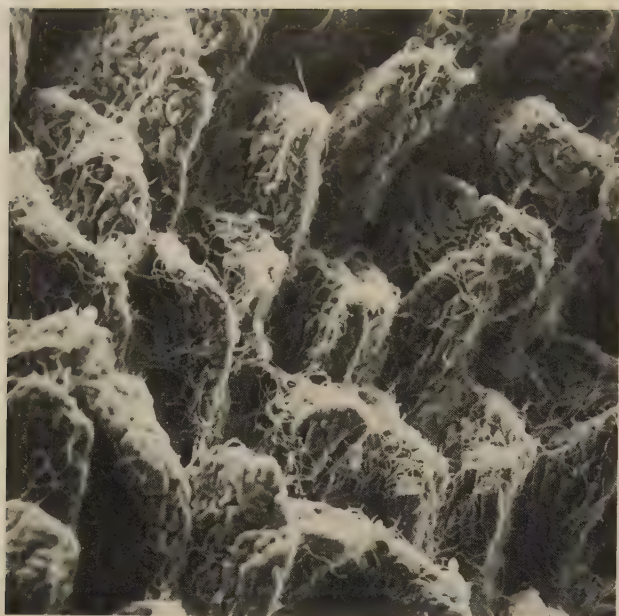


Fig. 237. Fract. irr. 1.8 Gy $\times$ 5. Massive attack by bacteria. Villi can hardly be seen. 190 $\times$.

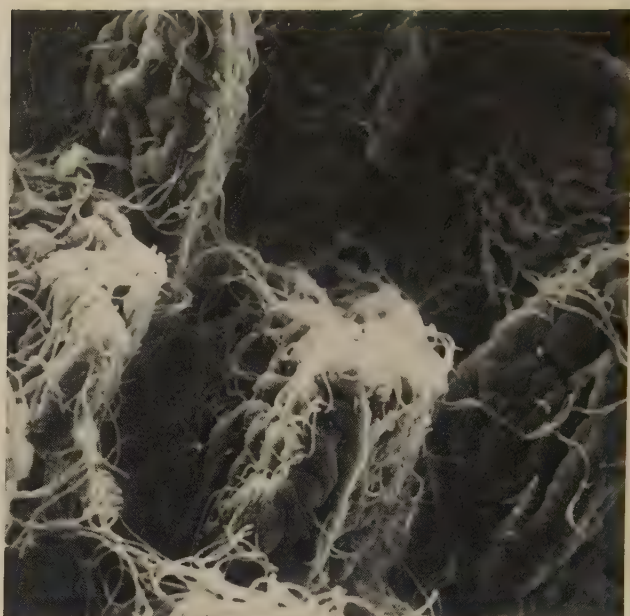


Fig. 238. Fract. irr. 1.8 Gy $\times$ 5. Retracted villi, swollen. Lots of bacteria. 470 $\times$.



Fig. 239. Fract. irr. 1.8 Gy $\times$ 5. Spherical, separated cells at the top of the villus. Bacteria. 1900 $\times$.



Fig. 240. Fract. irr. 1.8 Gy $\times$ 5. Many bacteria attacking at the same point. Note the mucinous granulae. Normal microvilli. 4700 $\times$.

Schedule: 1.8 Gy $\times$ 5, 2.2 Gy $\times$ 5. Metronidazole

Using cobalt-60 technique in the clinic, the results of pelvic irradiation are not good using daily doses of 2.2 Gy. Increased gastrointestinal symptoms are registered, which are hardly seen giving 2.0 Gy per day. In order to find out if small variations in daily doses could induce changes in the intestinal mucosa

seen by SEM, two other schedules were employed. In the first one, 1.8 Gy was given daily 5 times a week for three weeks, and in the other one, 2.2 Gy.

In this study it was not possible to find any difference in score number based on morphological reaction between the three fraction types used, 1.8 Gy, 2.0 Gy and 2.2 Gy per day. Some differences



Fig. 241. *Fract. irr. 1.8 Gy* $\times 5$. Metronidazole given. High, "clean" villi almost free from bacteria. 190 $\times$.



Fig. 242. *Fract. irr. 1.8 Gy* $\times 5$. Metronidazole given. Goblet cells up to the top. 470 $\times$.

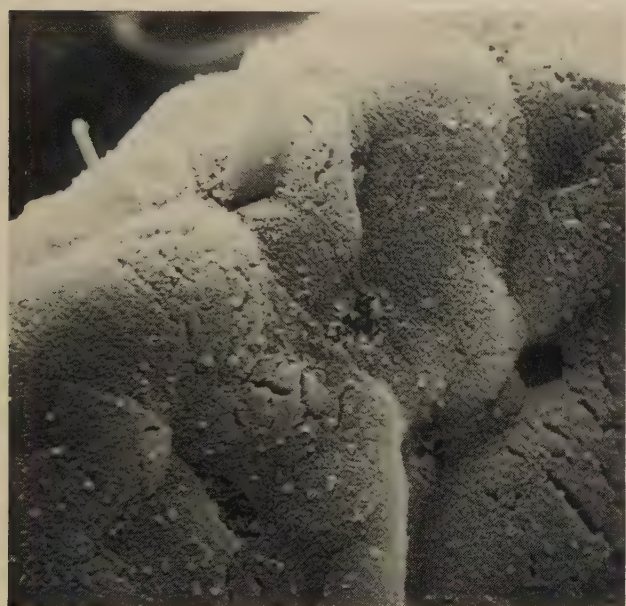


Fig. 243. *Fract. irr. 1.8 Gy* $\times 5$. Metronidazole given. Top of a villus. Even cell surface. Mucinous granulae. 1900 $\times$.



Fig. 244. *Fract. irr. 1.8 Gt* $\times 5$. Metronidazole given. Part of Fig. 242. Goblet cell. Sparse microvilli. 9000 $\times$.

may, however, be worth mentioning. After 5 F of 1.8 Gy the bacterial attack was more intensive than after 5 F of 2.2 Gy, and at the same time the Goblet cells did not seem to release their contents after the higher daily dose. In both fractionation schedules, no bacteria were seen and almost no oedema when metronidazole was given at a dose of 24 mg/rat/ day,

given continuously in the water during the whole irradiation period. The villi were high and narrow after metranidazole. The extrusion seen after 2.2 Gy/day could hardly be detected in the schedule with metronidazole. The conclusion drawn from this study is that metronidazole during 5 fractions of 1.8 and 2.2 Gy had a rather protective effect. The villi



Fig. 245. Fract. irr. 2.2 Gy $\times 5$. Swollen, retracted villi. Moderate attack by bacteria. 470 $\times$.



Fig. 246. Fract. irr. 2.2 Gy $\times 5$. Part of Fig. 001. Clean surface. Swollen cells. Closed Goblet cells. 2000 $\times$.

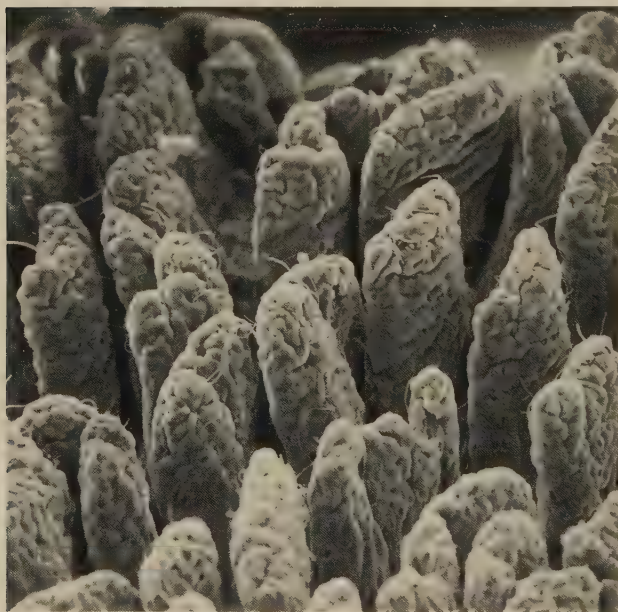


Fig. 247. Fract. irr. 2.2 Gy $\times 5$. Metronidazole given. Erected villi. Most bacteria are gone. 190 $\times$.

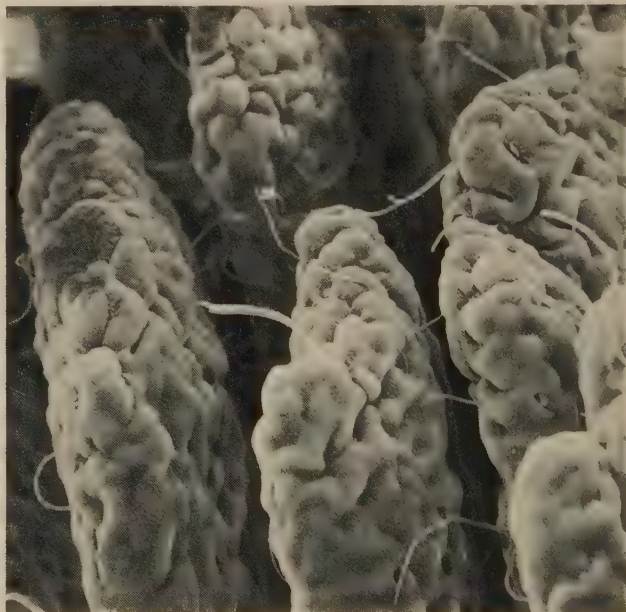


Fig. 248. Fract. irr. 2.2 Gy $\times 5$. Metronidazole given. The villi are less swollen. 470 $\times$.

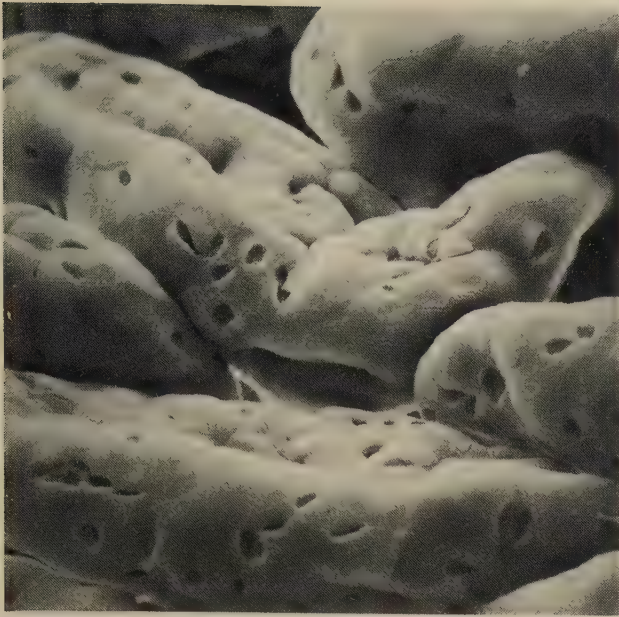


Fig. 249. *Fract. irr. 2 Gy* $\times 5$. Fasting animal. Swollen retracted villi. Clean surface. Goblet cells on the top. No bacteria. 470 $\times$.



Fig. 250. *Fract. irr. 2 Gy* $\times 5$. High magnification. Three closed Goblet cells. 4700 $\times$.

were almost like normal villi in fasting animals. The absence of secretion granulae and continued attack by bacteria may be a partial sign of effect on the function of the cells. Irradiation using the conventional schedule, 2.0 Gy 5 times a week, in fasting animals, resulted in another reaction. There were low, swollen villi with absolutely clean surfaces and Goblet cells in large amounts all the way up to the extrusion zone, without emptying. It would appear from Fig. 249, that the extrusion process is very low, which could be explained by the fasting condition but not by the irradiation. The microvilli are normal and the bacteria do not appear as usual after repeated doses or high single doses. Thus the fasting in itself must be a stronger factor in the symbiotic activity than irradiation.

Animals fasting for 5 days living only on water, compared to animals given, as previously mention-

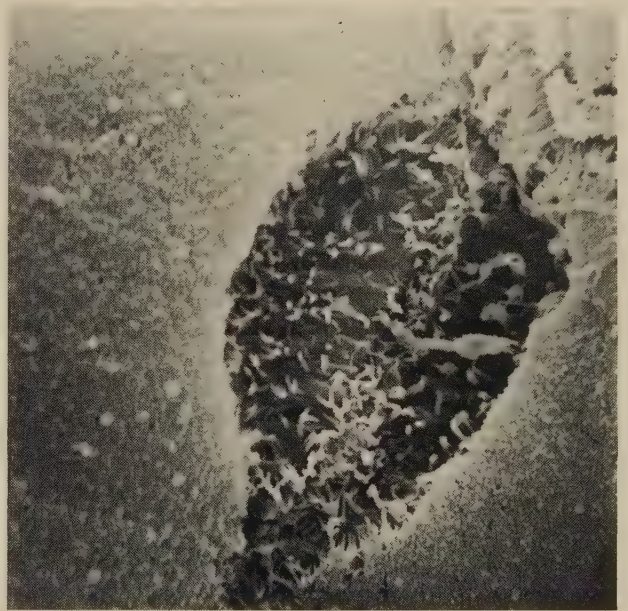


Fig. 251. *Fract. irr. 2 Gy* $\times 5$. Closed Goblet cell. 9000 $\times$.

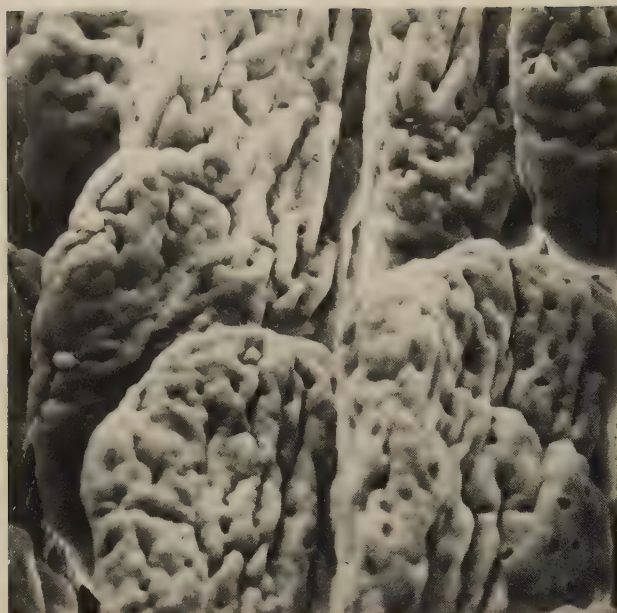


Fig. 252. Control. Non-irradiated villi in fasting animal. 470 ×.



Fig. 253. Control. Enlargement of Fig. 252. Extrusion zone of a villus. 1900 ×.



Fig. 254. Control. Non-irradiated villi in fasting animal. Metronidazole given. Swollen villi. Only moderate folds. 470 ×.



Fig. 255. Control. Enlargement of Fig. 254. Metronidazole given. No difference from Fig. 253. 1900 ×.

ed, metronidazole every day for 5 days in another fasting group did not show any difference at all at higher magnification of the villi and the extrusion

zone. As a whole, the villi were more swollen, with no pattern of folds, as seen in the control group. The Goblet cells on the top appear to be unemptied.

Fract. irr. 2 F/week

Schedule: 2 F/week – 5 Gy $\times$ 2/week

5 Gy was given usually on Monday and Thursday during the overall time of three weeks. The weight of the rats was controlled daily. As will be seen from the weight curve, and also the score curve, the reaction after each fraction seems to have been almost repaired during the 72 and 96 hours interval. The schedule differs from 5 F/week. Bacteria and Goblet cell activity are absent. Microvilli may be more affected, but to a degree that can hardly be measured.

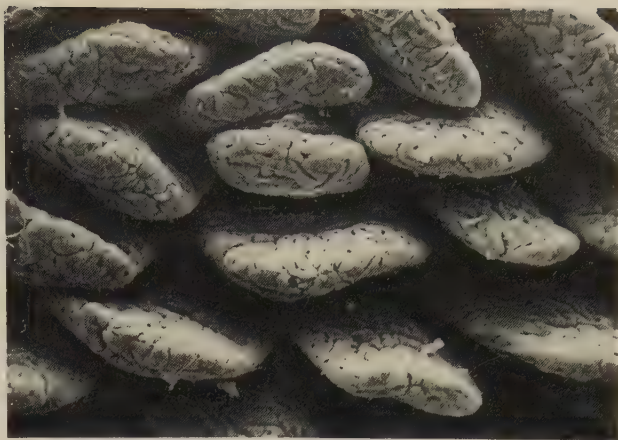


Fig. 256. Fract. irr. 2 F/week – 5 Gy $\times$ 2. Slightly swollen villi. No bacteria. 190 $\times$.



Fig. 257. Fract. irr. 2 F/week – 5 Gy $\times$ 2. Goblet cells, closed, on the tops of the villi. Enlargement from Fig. 256. 900 $\times$.



Fig. 258. Fract. irr. 2 F/week – 5 Gy $\times 2$. Swollen epithelial cells on the top of a villus. Closed Goblet cells. Microvilli diverge on all cells. No bacteria. 3700 $\times$.



Fract. irr. 2 F/week – 5 Gy $\times 2$

Villi are only slightly swollen. Almost no extrusion can be seen. No bacteria. Goblet cells migrate up to the top without being emptied. The epithelial cells are swollen but not separated from each other. The microvilli are sparse on all cells. A few mucinous granule can be seen. Fig. 256–Fig. 259.

Fig. 259. Fract. irr. 2 F/week – 5 Gy $\times 2$. A closed Goblet cell. Microvilli are swollen at the tips and diverge. 9000 $\times$.

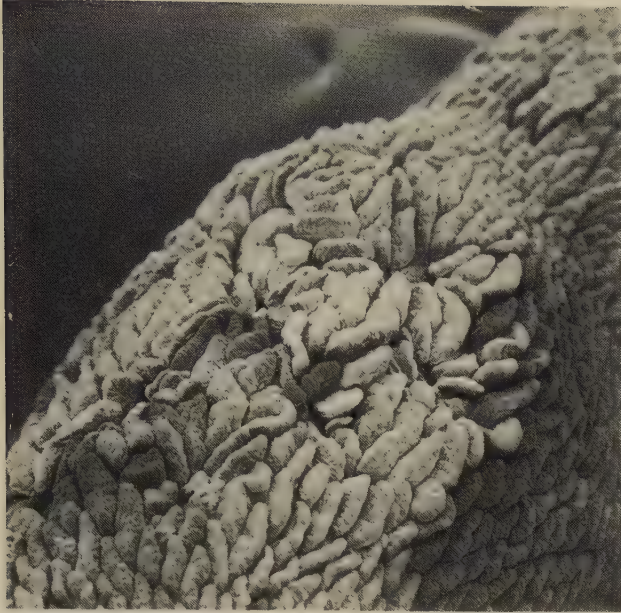


Fig. 260. Fract. irr. 2 F/week - 5 Gy $\times$ 3. The nodules of a Peyer's patch have diminished and are almost hidden between the villi. 45 $\times$.

Fract. irr. 2 F/week - 5 Gy $\times$ 3

Villi are retracted and swollen. No bacteria. Goblet cells are seen, closed, at the tops of the villi. Still there are mucinous granulae on the surface. The

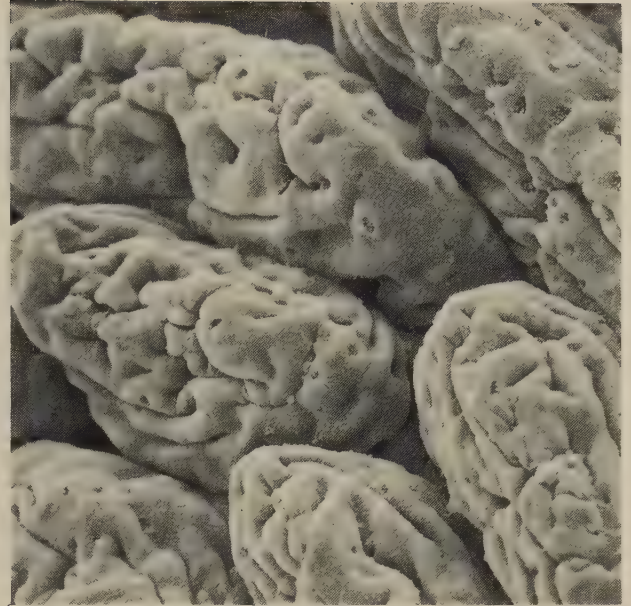


Fig. 261. Fract. irr. 2 F/week - (5 Gy $\times$ 2) $\times$ 3. Villi are slightly swollen. No extrusion. Goblet cells on the top. A few bacteria. 600 $\times$.

epithelial cells are spherical in form. As seen in Fig. 260, Peyer's patch has diminished and can hardly be seen between the villi.



Fig. 262. Fract. irr. 2 F/week - 5 Gy $\times 3$. Villi are swollen and retracted. Goblet cells are seen on the tops of the villi, closed. No bacteria. In the center a shrunk nodule of a Peyer's patch. 375 $\times$.



Fig. 263. Fract. irr. 2 F/week - 5 Gy $\times 3$. Enlargement of a top of a villus, swollen. No extrusion. Closed Goblet cells. Polygonal epithelial cells. 3700 $\times$.

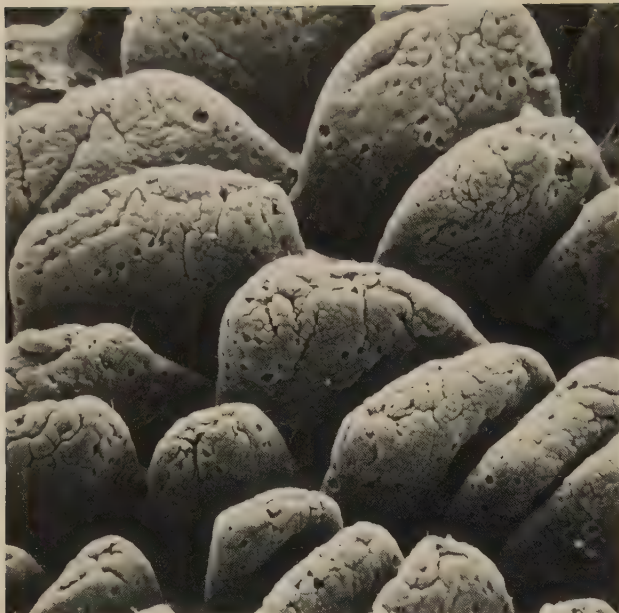


Fig. 264. Fract. irr. 2 F/week – (5 Gy $\times$ 2) $\times$ 2. Swollen villi. Some have pyramidal form. No bacteria. No extrusion. 190 $\times$.

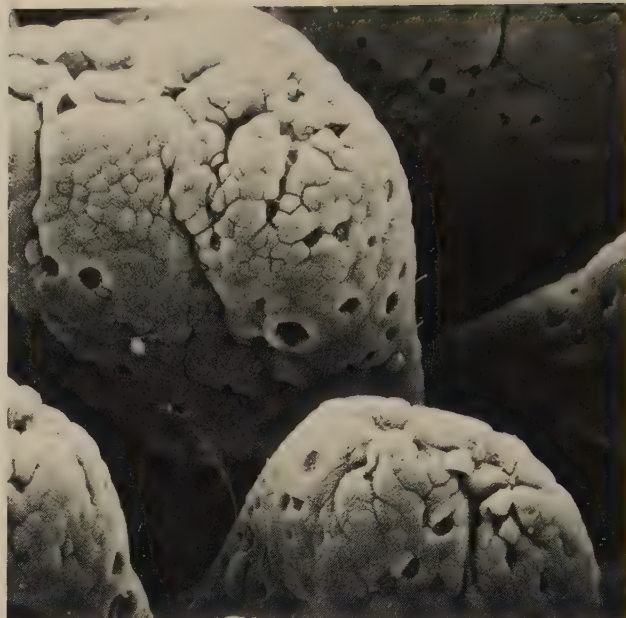


Fig. 265. Fract. irr. 2 F/week – (5 Gy $\times$ 2) $\times$ 2. The large Goblet cells are seen all over the swollen villi. 570 $\times$.



Fig. 266. Fract. irr. 2 F/week – (5 Gy $\times$ 2) $\times$ 2. Half spherical surfaces of the epithelial cells. No bacteria. Picture from the top of a villus. 1900 $\times$.

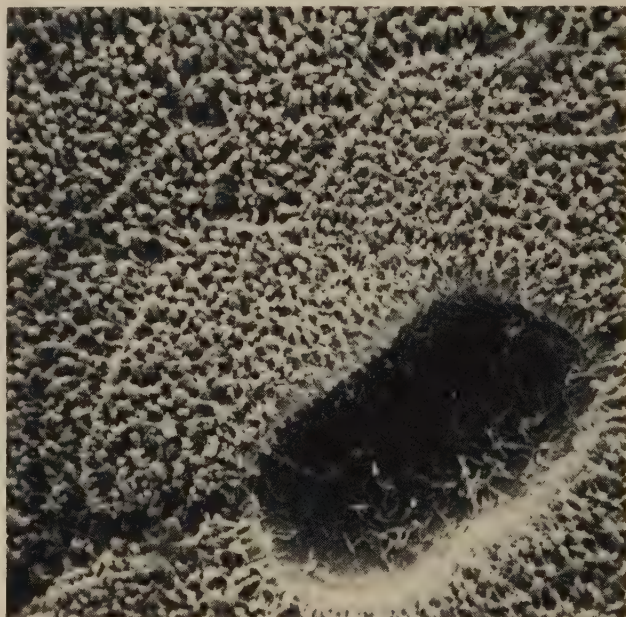


Fig. 267. Fract. irr. 2 F/week – (5 Gy $\times$ 2) $\times$ 2. Closed Goblet cell with a few microvilli on the membrane. Microvilli on the epithelial cells diverge and are perhaps shorter than normal. 4700 $\times$.

Fract. irr. 2 F/week – (5 Gy $\times$ 2) $\times$ 2

Villi are swollen. Only a few have a pyramidal form. No bacteria. The epithelial cells are swollen and the

microvilli on the surface are sparse and may be shorter than normal. The Goblet cells are closed. No mucinous granulae. Figs. 264–267.



Fig. 268 (a). Fract. irr. 2 F/week – (5 Gy × 2) × 3. Villi are slightly swollen. No extrusion. Goblet cells on the top. A few bacteria. 470 ×.



Fig. 268(b). Fract. irr. 2 F/week – (5 Gy × 2) × 3. 800 ×.

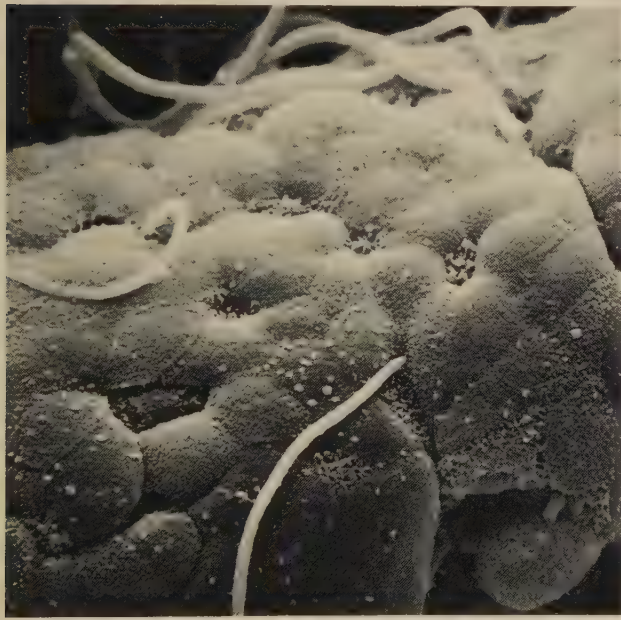


Fig. 269 (a). Fract. irr. 2 F/week – (5 Gy × 2) × 3. Top of a villus. No extrusion. The Goblet cells seem to be closed. Still there are mucinous granulae on the cell surfaces. A few bacteria. 1900 ×.

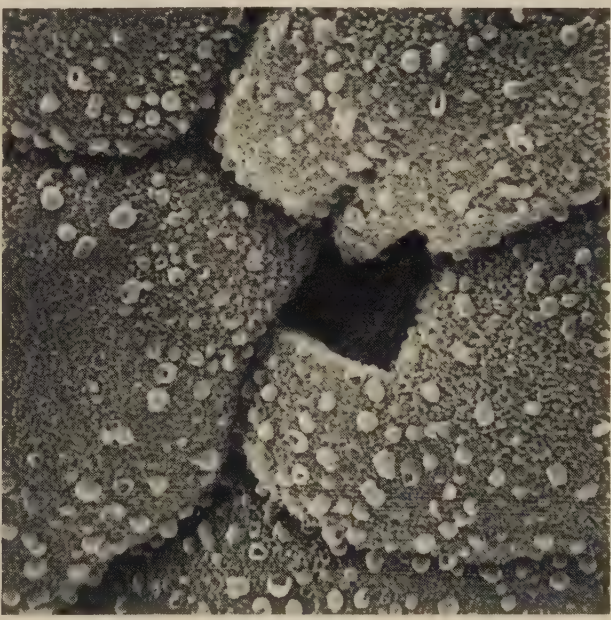


Fig. 269(b). Fract. irr. 2 F/week – (5 Gy × 2) × 3. Mucinous granulae on the surface of the epithelial cells. 5000 ×.

Fract. irr. 2 F/week – (5 Gy × 2) × 3

Villi are only slightly swollen. Almost no extrusion is seen. Goblet cells are seen on the tops of the villi, closed. Still there are mucinous granulae on the

surface. On some villi the microvilli diverge, on others microvilli are not far from normal. On some villi a few bacteria can be seen. Figs. 268 (a)–269 (a).

Fract. irr. 15 F/week

Schedule: 15 F/Week (1 Gy × 3)/day, 5 days/week

1 Gy was given at 8.00 o'clock a.m., 4.00 o'clock p.m. and at midnight 5 days a week for three weeks. This schedule was doubled, and in the second one, metronidazole was from the beginning added to the water supply during three weeks. All rats in both series had, as in the other schedules, free access to water and pellets. The dose of metronidazole was 48 mg per 24 hours to two rats. Two rats were kept in each cage and two rats were removed and sacrificed every 24 hours during the three weeks. Due to the multifractionation with only 8 hours between the doses of 1 Gy day and night, the rats slept from 30–120 minutes after the anaesthesia at each dose.

This could result in a lesser food-intake and a greater decrease in weight-reduction than seen in other regimes. The three fractions simulated only by anaesthesia gave an increased reduction in weight in non irradiated animals. During the three weeks there were bacteria all the time, indicating that the rats were eating.

Some examples are shown from this schedule with and without metronidazole.

After 9 fractions ($Gy \times 3$) × 3. 24 hours after three days of irradiation the villi cannot be separated by appearance from normal eating animals. The villi are retracted, swollen with clear folds and a moderate degree of bacteria. In the group given metronidazole no bacteria can be seen and the villi are not swollen.



Fig. 270(a). *Fract. irr. (1 Gy × 3) × 3*. Normal villi, retracted. A moderate number of bacteria. 470 ×.



Fig. 270(b). *Fract. irr. (1 Gy × 3) × 3*. Metronidazole. No bacteria. The villi are not swollen. 470 ×.



Fig. 271(a). *Fract. irr. (1 Gy × 3) × 6*. Villi with bacteria. 190 ×.

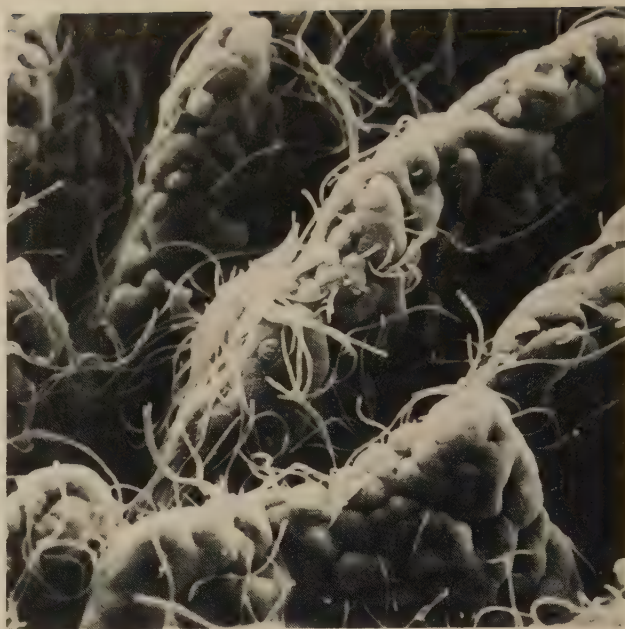


Fig. 271(b). *Fract. irr. (1 Gy × 3) × 6*. Long ridges of the villi. Part of Fig. 271(a). 470 ×.



Fig. 272. *Fract. irr. (1 Gy × 3) × 6*. Swollen, separated cells on the top. Diverging microvilli. Intensive attack of bacteria. 1900 ×.



Fig. 273. *Fract. irr. (1 Gy × 3) × 6*. Microvilli are diverging and of different length. 4700 ×.

After 18 fractions, $(1 \text{ Gy} \times 3) \times 6$, the villi are not far from normal. Some, but only some, have a triangular form and are moderately swollen. A heavy attack by bacteria is seen on the upper third part. On the other hand, high magnification reveals spherical cells with sparse microvilli and an irregular

cell arrangement. After the same number of fractions with metronidazole almost all bacteria of the fusiform anaerobe type have disappeared. The villi are less active on the surface without separated cells and with a tight microvilli surface.



Fig. 274. Fract. irr. (1 Gy $\times$ 3) $\times$ 6. Metronidazole. Almost no bacteria. Almost no cells in extrusion. No separated, spherical cells. More "quiet" surface than without metronidazole. 900 $\times$.

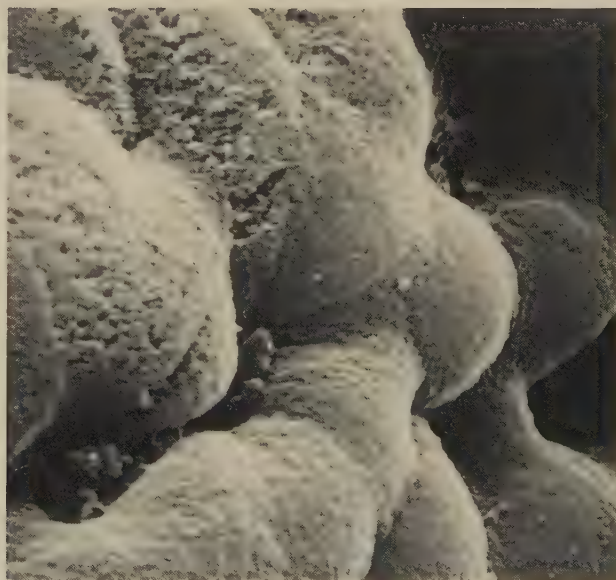


Fig. 275. Fract. irr. (1 Gy $\times$ 3) $\times$ 6. Metronidazole. Side of a villus. No bacteria, swollen cells, normal microvilli. 4700 $\times$.



Fig. 276. *Fract. irr. (1 Gy $\times$ 3) $\times$ 7*. View showing triangular villi, increased extrusion and a moderate degree of bacteria. 200 $\times$.

After 21 fractions, (1 Gy $\times$ 3) $\times$ 7, the villi have changed to a distinct triangular form. There is cell-extrusion at the tops and fewer bacteria. The

microvilli are even here spread from each other just as was also seen after 18 fractions.



Fig. 277. Fract. irr. (1 Gy × 3) × 7. Upper part of the villus side. Separated epithelial cells with diverging microvilli on all surfaces. 1900 ×.



Fig. 278. Fract. irr. (1 Gy × 3) × 7. Four cells with a rough microvilli-covering. 9000 ×.

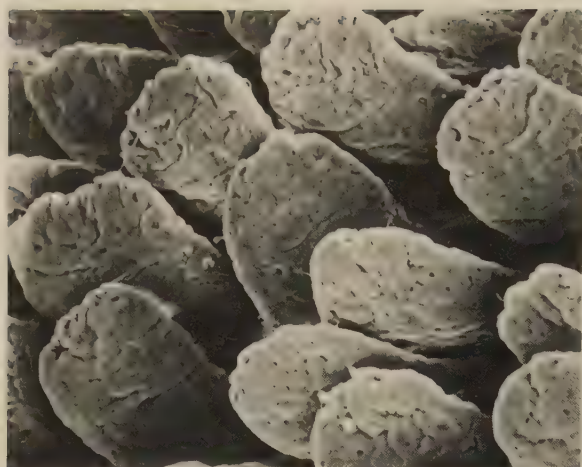


Fig. 279. *Fract. irr. (1 Gy $\times$ 3) $\times$ 10. Metronidazole.* Thin, slender villi, triangular or fingerlike. No bacteria. Goblet cell openings. 190 $\times$.

After 30 fractions (1 Gy $\times$ 3) $\times$ 10, without metronidazole, the villi have not quite the typical triangular form, which means a little lower score number, but they still have bacteria. In pictures from rats having metronidazole at the same dose, the villi are gracile with no swelling and without bacteria. Goblet cells can be seen all the way up to the top, unemptied.

Fig. 280. *Fract. irr. (1 Gy $\times$ 3) $\times$ 10. Metronidazole.* Higher magnification. Again more quiet surface. Not much swollen cells. Hardly any extrusion. 600 $\times$.



After 45 fractions, after 3 weeks, $(1\text{ Gy} \times 3) \times 15$, about the same kind of villus configuration as after 30 fractions is seen. Narrow tops with bacteria and non-empty Goblet cells. In high enlargement the epithelial cells and microvilli are swollen, sparse, to the same degree as after 21 F. The metronidazole group at 45 F and three weeks shows a more inactive picture with triangular villi having none or only a

few visible extrusion cells at the tops. There are no bacteria. The epithelial cells can easily be distinguished from each other, some have the same sparse microvilli as in the series without metronidazole. Cells have closely packed microvilli as in the normal cells. In low magnification some Goblet cells are seen, but in high magnification no mucinous granulae are visible. The difference between the effects

Fig. 281. *Fract. irr. $(1\text{ Gy} \times 3) \times 15$* . Fingerlike villi with narrow tops, where the bacteria are attacking. Goblet cell openings on the vertex of the villi. 1000 $\times$.





Fig. 282. *Fract. irr. (1 Gy × 3) × 15*. Bulky, swollen cells. Goblet cells/epithelial cells is close to 1:2. 1900 ×.



Fig. 283. *Fract. irr. (1 Gy × 3) × 15*. Side of villus. Bacteria. Microvilli rather tightly packed. 1900 ×.



Fig. 284. *Fract. irr. (2 Gy × 3) × 15*. In high magnification the microvilli are diverging. 4700 ×.

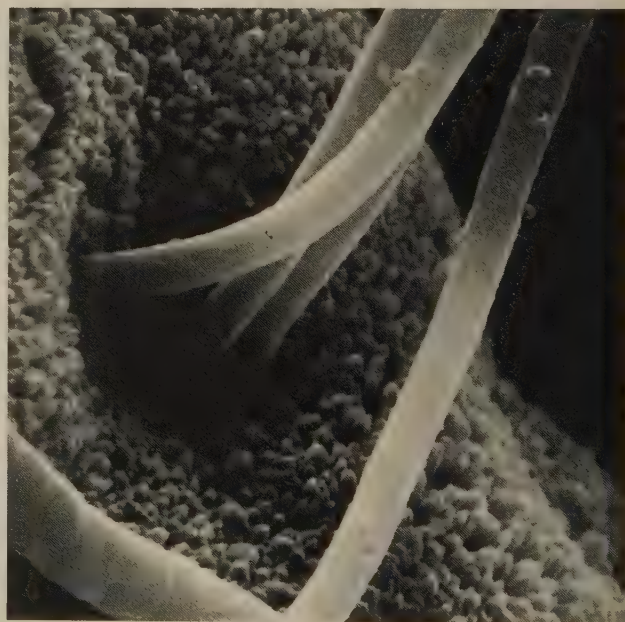


Fig. 285. *Fract. irr. (1 Gy × 3) × 15*. Swollen microvilli tips with about twice the normal diameter. 9000 ×.



Fig. 286. Fract. irr. (1 Gy $\times$ 3) $\times$ 15. Metronidazole. Slender villi, triangular in form. Irregular form of the cells. No extrusion. No bacteria. 900 $\times$.

is minor and difficult to express in score-steps. The most obvious sign at 45 F is the lack of activity in the group with metronidazole. There are no bacteria, no active Goblet cells and the microvilli are only

slightly spread. This could be registered as a protective effect. Other possibilities cannot be eliminated. See the discussion.



Fig. 287. *Fract. irr. (1 Gy × 3) × 15. Metronidazole.* Regular pattern as in a fasting animal with narrow tops. No bacteria. 190 ×.



Fig. 288. *Fract. irr. (1 Gy × 3) × 15. Metronidazole.* At this moment there are no extruding cell. Large marked epithelial cells only slightly swollen. See Fig. 286. 470 ×.



Fig. 289. *Fract. irr. (1 Gy × 3) × 3. Metronidazole.* Side of a villus. Clean but with some demarcated cells with more diverging microvilli. The side cells are swollen. 1900 ×.



Fig. 290. *Fract. irr. (1 Gy × 3) × 15. Metronidazole.* Close up from Fig. 289.

Fract. irr. 3 F/week

Schedule: 3F/week (4 Gy + 3 Gy + 3 Gy) per week

In the clinic, irradiation of the pelvis, using this schedule, resulted in serious primary reactions, in some cases requiring surgery (*Johnsson*, personal communication, 1975). Three fractions a week is otherwise an acceptable regime as long as the total dose is reduced. With lower fraction size, *Marcial and Bosch* (1968) reported no difference in reactions between schedules of five or three fractions a week, given as 1.5 Gy and 2.5 Gy, respectively, per fraction. It has been considered desirable to study the effect by simulating this “odd” regime. During the overall time of three weeks, two rats were sacrificed 24 hours after each dose. The post-irradiation period was controlled during 13 days.

Fractions were given at an interval of 48 hours,

with a resting period of 72 hours. In crypt studies, the maximum effect of single doses of this range is established at 48 hours (*van der meer fieggen*, 1973). The proliferation starts after 2 days (*Devik*, 1971). Between 2.5 and 4 days an intense proliferation in the crypts is going on, the so-called “over-shooting” phenomenon. Each dose in this fractionation regime is thus given during a critical period in the crypts.

3F/week—4 Gy—1 fraction

24 hours after the first irradiation no particular reaction of the villus was seen. Some bacteria can be detected, as well as Goblet cells, not emptied even at the tops of the villi. In high magnification the microvilli are diverging in a way shown in the 5 F/week-schedule.

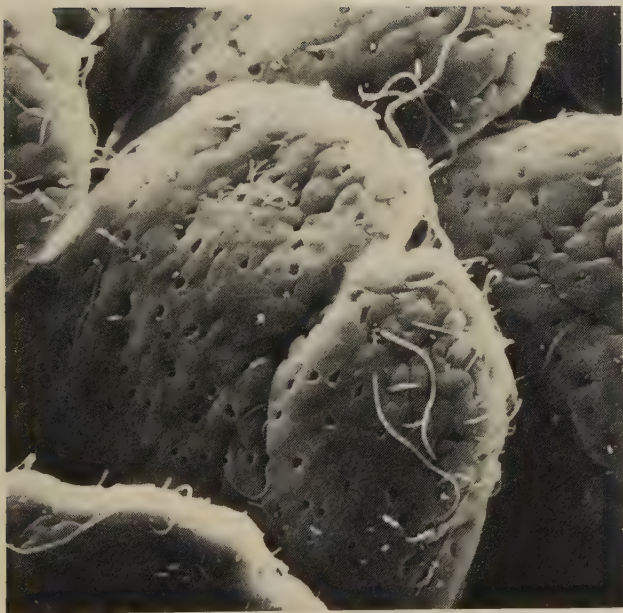


Fig. 291. Fract. irr. 3 F/week – 1 fraction. Swollen villi. Hardly any folds. Goblet cells, closed, up to the top. Moderate number of bacteria. 470 ×.

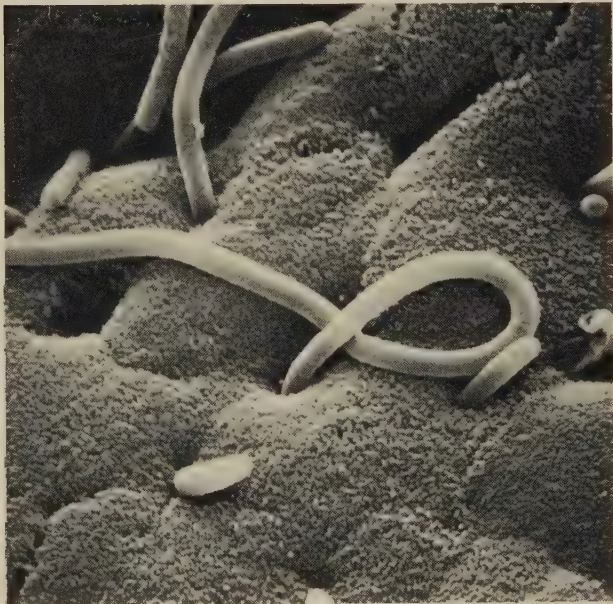


Fig. 292. Fract. irr. 3 F/week – 1 fraction. Bacteria. Diverging microvilli on every epithelial cell. 4700 ×.

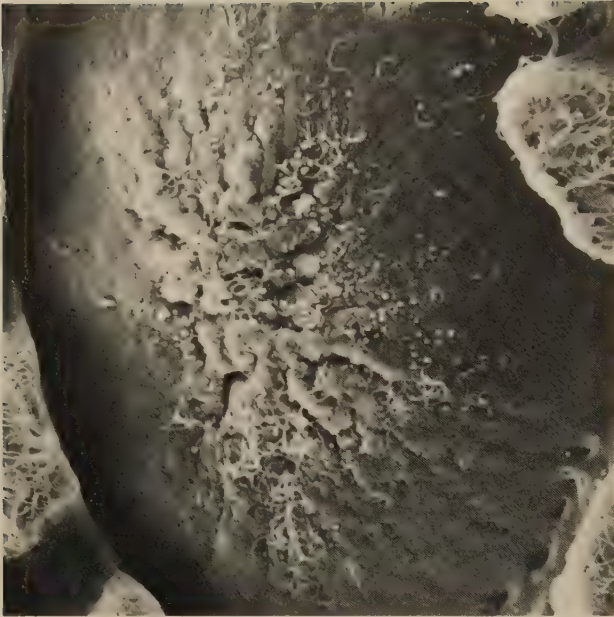


Fig. 293. *Fract. irr. 3 F/week – 3 fractions. Peyer's patch. Bacteria in the center. Leakage of cells. Bacteria on the tops of the villi. 190 ×.*

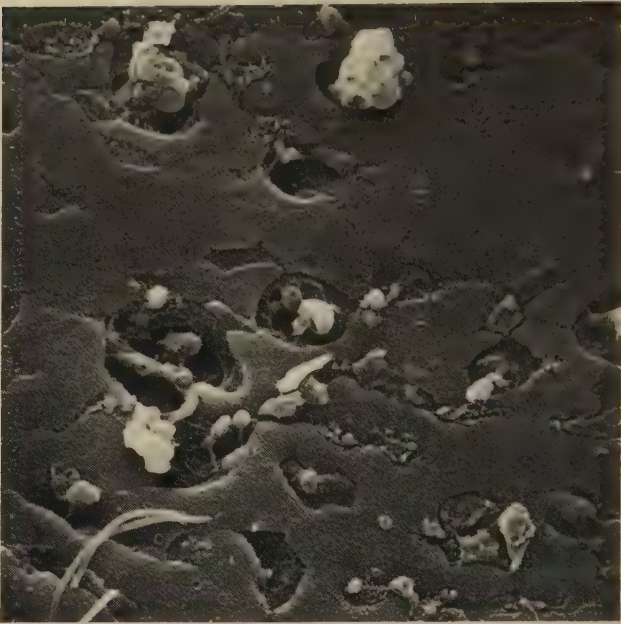


Fig. 294 (a). *Fract. irr. 3 F/week – 3 fractions. High magnification of Fig. 293. Cells are penetrating the surface. The cells cannot be identified. 1000 ×.*

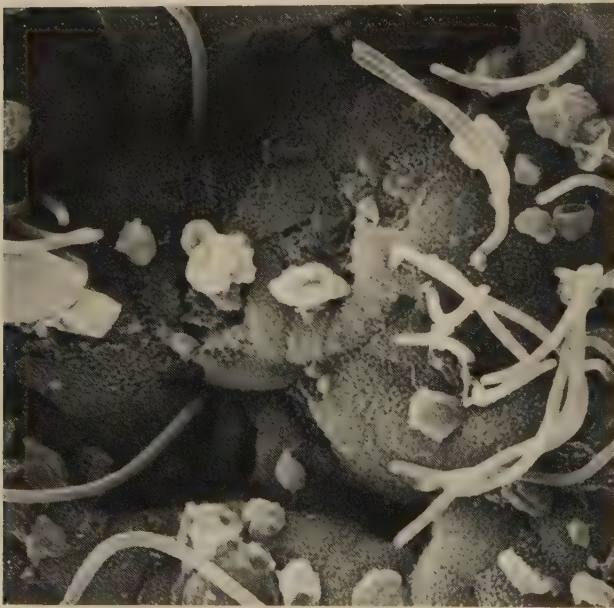


Fig. 294(b). *Fract. irr. 3 F/week – 3 fractions. Enlargement of Fig. 293, the central area. Leakage of cells and bacteria on the ridges, which converge to the center of Peyer's patch. 1900 ×.*

3 F/week–10 Gy–3 fractions

In the first week, after three fractions, the villi start to change the form into a pyramidal-shaped type. More cells are extruded than normally and the attack by bacteria is massive. An increased number of bacteria also attack the center of the Peyer's patch and many lymphoid cells leak through the peripheral area (Fig. 293 and Fig. 294).

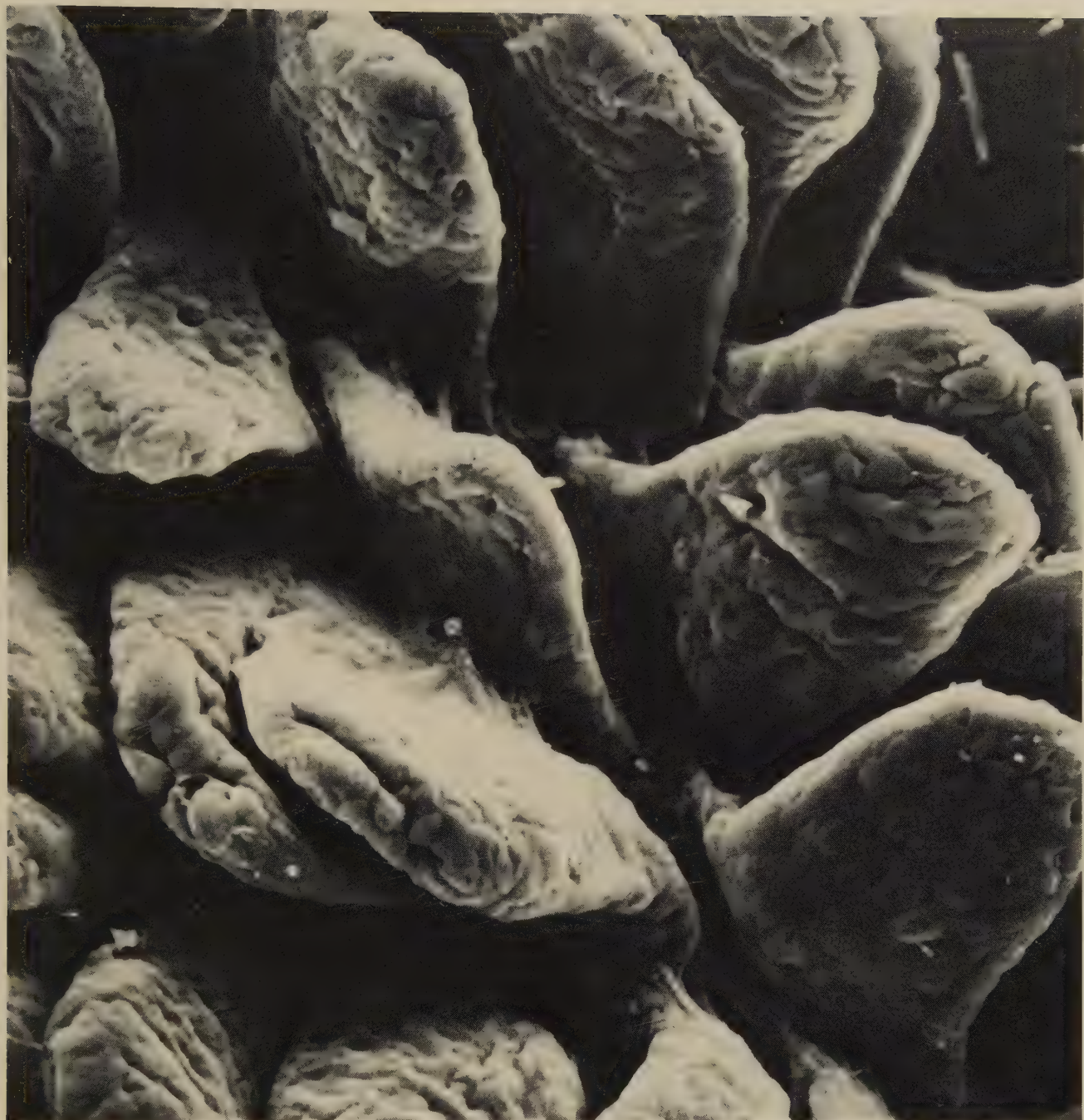


Fig. 295. Fract. irr. 3 F/week – 7 fractions. Slender, narrow villi. No bacteria. Pyramidal form. Closed Goblet cells on the side walls. 370 $\times$.

3 F/week–24 Gy–7 fractions

Villi are high and have a pyramidal form. Almost no extrusion is seen and bacteria have disappeared. On the side walls large Goblet cells in increased number are seen. High magnification of these cells confirm

the closed openings (Fig. 296 and Fig. 297). The epithelial cells are not swollen, but have an irregular outline. All microvilli are diverging and seem to be shorter than normal. No mucinous granulae are seen.

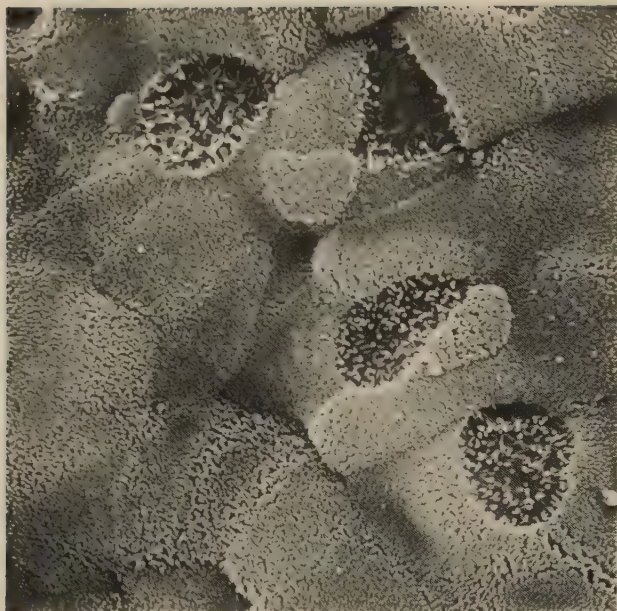


Fig. 296. Fract. irr. 3 F/week – 7 fractions. Side of a villus. Large closed Goblet cells. No mucinous granulae. Diverging microvilli on the epithelial cells. 1900 ×.

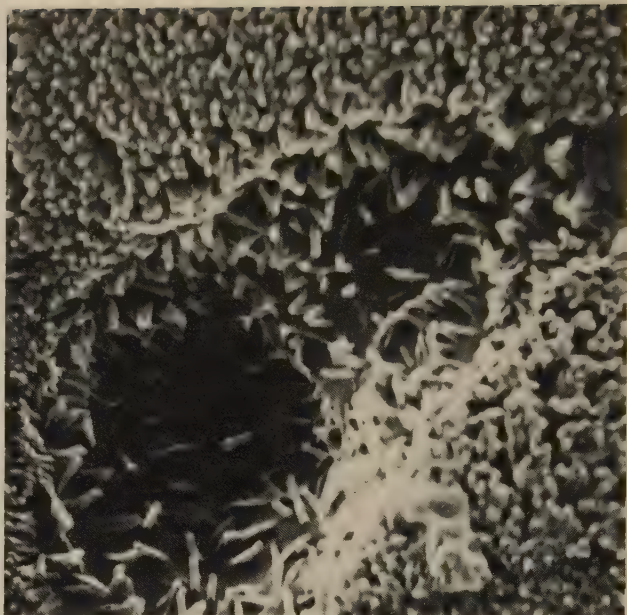


Fig. 297. Fract. irr. 3 F/week – 7 fractions. High magnification of a Goblet cell, closed and with a small number of microvilli on the membrane. Diverging microvilli on the surrounding cells 9000 ×.



Fig. 298. Fract. irr. 3 F/week – 9 fractions. Finger-like villi. Swollen tops. No extrusion. No bacteria. The bottom between the villi can be seen as well as the ridges between the villi. 190 ×.



Fig. 299. Fract. irr. 3F/week – 9 fractions. Finger-like villi. Folds more or less expressed. A few bacteria on the lower part of the villi. 470 ×.

3 F/week–(30 Gy–9 fractions)–3 days

At three days after total dose, 30 Gy, the villi are finger-like, swollen at the tops with hardly any visible extrusion. The epithelial cells are plain and of various outline. Some are narrow, some are polygonal. Big closed Goblet cells are clearly seen up to the top (Fig. 300). The microvilli have the same appearance as after 7 fractions. The scoring of the average reaction, taking into consideration the total picture, is similar to the reaction in the schedule with 1 F/week–(9 Gy at 2 fractions)–3 days later.

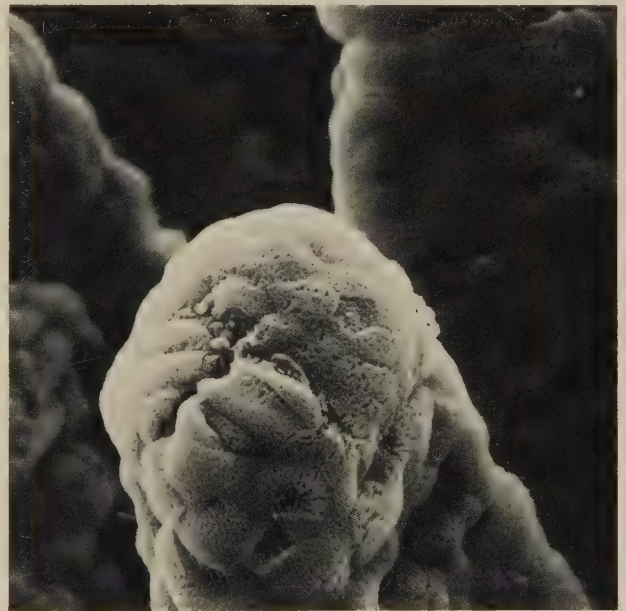


Fig. 300. Fract. irr. 3 F/week – 9 fractions. Top of a villus. Large Goblet cell, closed at the top. Irregular form of the epithelial cells. 900 ×.



Fig. 301. Fract. irr. 3 F/week - 9 fractions. 7 days later. Villi with long ridges. No bacteria. Many cells in extrusion. 190 ×.



Fig. 302. Fract. irr. 3 F/week - 9 fractions. 7 days later. Top of a villus. 2-3 bacteria. Deep folds. Magnification of Fig. 301. 900 ×.

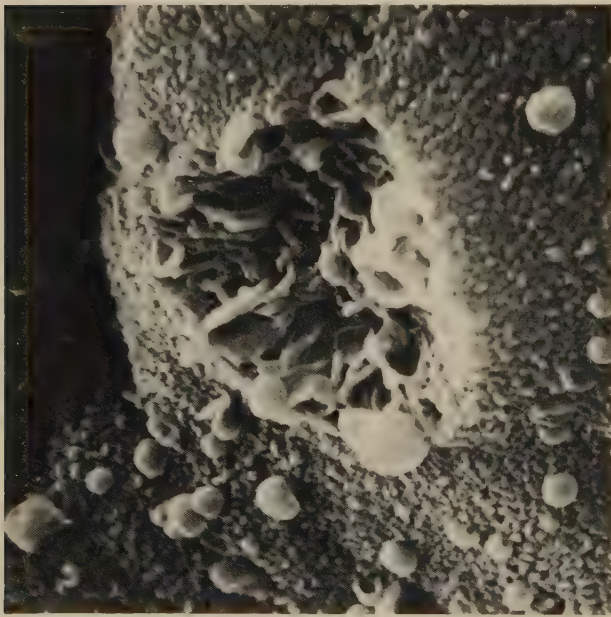


Fig. 303. Fract. irr. 3 F/week - 9 fractions. 7 days later. Wide open Goblet cell after emptying. Microvilli normal. 9000 ×.

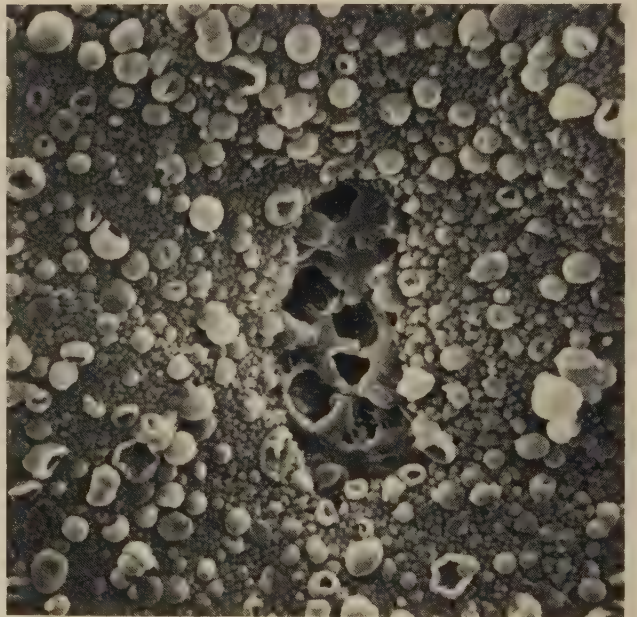


Fig. 304. Fract. irr. 3 F/week - 9 fractions. 7 days later. Empty framework of a Goblet cell, mucinous granulae on the cells. Normal microvilli. 10,000 ×.



Fig. 305. *Fract. irr. 3 F/week – 9 fractions. 13 days later. Normal Peyer's patch and villi. 90 ×.*

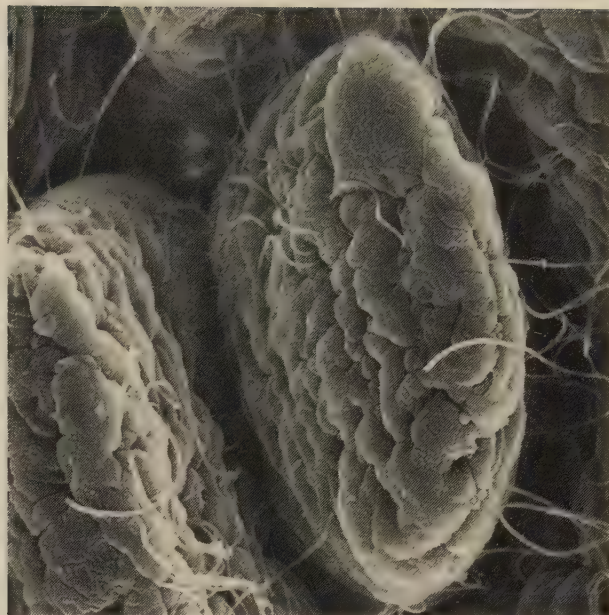


Fig. 306. *Fract. irr. 3 F/week – 9 fractions. 13 days later. Swollen villi. Bacteria. 500 ×.*

3 F/week–(30 Gy–9 fractions)–7 days

At 7 days after completed irradiation the villi are leaf-formed with a long ridge. The extrusion is more active. Lots of Goblet cells empty their mucinous granulae explosively on the cell surfaces. Microvilli are diverging only on the upper part of the villi. Bacteria are attacking at the tops again (Fig. 011 and Fig. 014).

3 F/week–(30 Gy–9 fractions)–13 days

The villi and Peyer's patch seem to be almost normal. As seen villi and epithelial cells are swollen. Mucinous granulae can be seen everywhere on the surface. The picture shows a morphologically normal villus with a high activity (Fig. 306 and Fig. 307). In conclusion this schedule resulted in a more advanced reaction than the other regimes, except 1 F/week, maybe due to the condition in the crypts when each fraction is given, as discussed earlier.

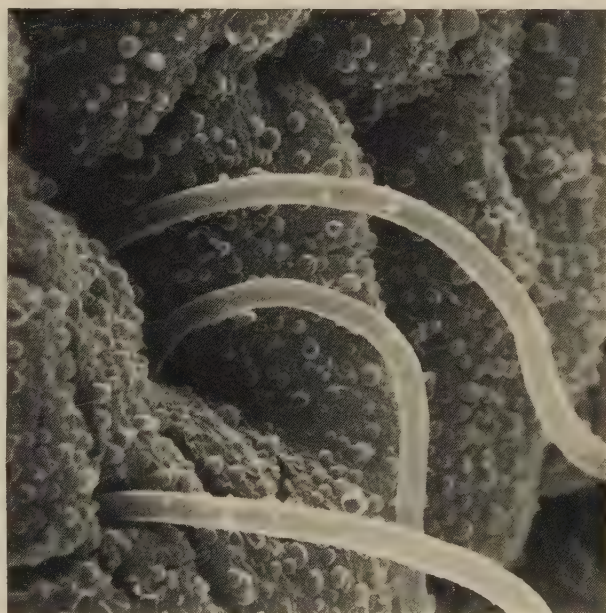


Fig. 307. *Fract. irr. 3 F/week – 9 fractions. 13 days later. High activity. Swollen, half spherical epithelial cells. Large amounts of mucinous granulae on the surface. Attack by bacteria on these cells on the side of a villus. 5000 ×.*

Fract. irr. 1 F/week

Fract. irr. (9 Gy $\times$ 1) – 3–7 days

A high dose of 9 Gy was given each Monday during three weeks. Specimens were examined especially after 72 hours, where the maximum reaction of a single dose of 9 Gy occur and after 7 days, the day when the next fraction was given. The total time is 15 days and the total dose 27 Gy. The repair after total dose was followed up to 14 days after irradiation. The result after one fraction, 9 Gy, is presented in Chapter VII: Single dose irradiation.

Fract. irr. (9 Gy $\times$ 2) – 3 days

The villi are finger-like, resulting in a sparse distribution. The tops are swollen, with extrusion in the center. The shapes of the epithelial cells differ, usually they are big with marked borders and a convex surfaces, on which the microvilli are shorter than normal, more or less sparse and with adhering swollen tops. No bacteria can be seen.

Fig. 308. Fract. irr. 9 Gy $\times$ 2 – 3 days. Finger-like villi. Irregular pattern. No bacteria. 370 $\times$.





Fig. 309. Fract. irr. 9 Gy $\times 2-3$ days. Two villi enlarged from Fig. 308 slightly folded with convex surface of the epithelial cells. 900 $\times$.



Fig. 310. Fract. irr. 9 Gy $\times 2-3$ days. Top of a villus in Fig. 309. 1900 $\times$.



Fig. 311. Fract. irr. 9 Gy $\times$ 2 – 3 days. Top of a villus. Different kinds of cell surfaces with irregularly distributed microvilli. No Goblet cells. No bacteria. 3700 $\times$.



Fig. 312. Fract. irr. 9 Gy $\times$ 2 – 3 days. Lateral part of a villus (functional compartment). Swollen cells. 1900 $\times$.



Fig. 313. Fract. irr. 9 Gy $\times$ 2 – 3 days. Enlargement of Fig. 312. Microvilli short and sparse 4700 $\times$.



Fig. 314. Fract. irr. 9 Gy $\times$ 2 – 3 days. Enlargement of Fig. 313. Short microvilli with adherent tops. 9000 $\times$.

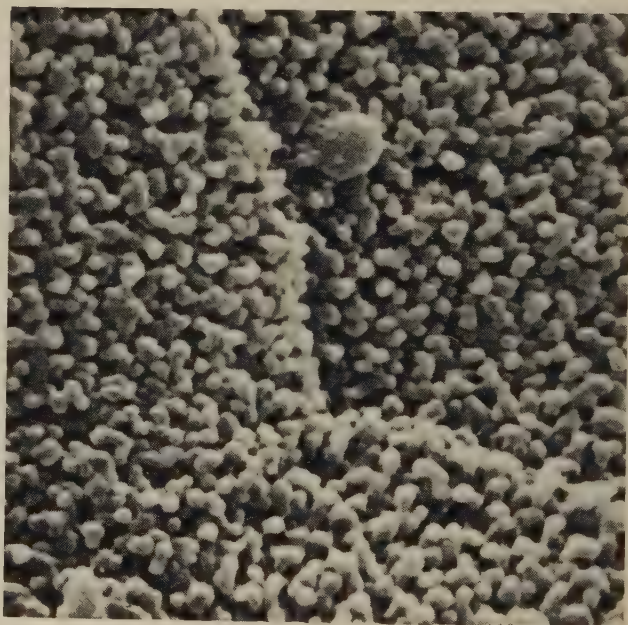


Fig. 315. Fract irr. 9 Gy $\times$ 2 – 3 days. High magnification of Fig. 313. Border between 3 cells. Swollen tips of the microvilli. 19,000 $\times$.

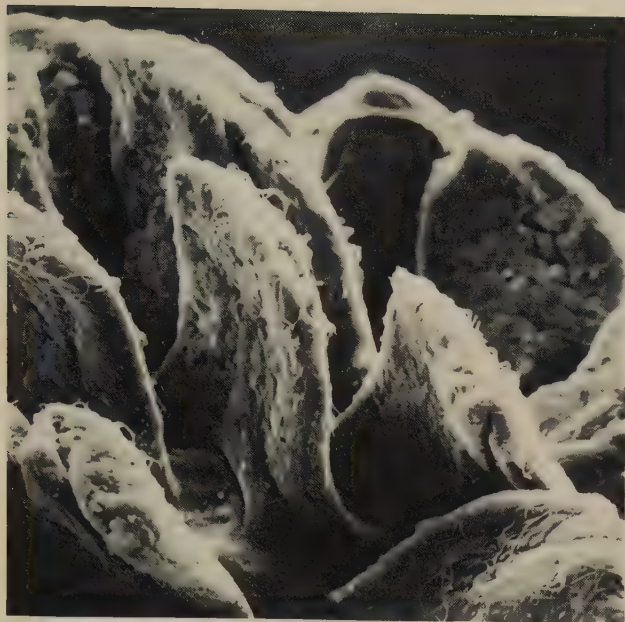


Fig. 316. *Fract. irr. 9 Gy $\times$ 2-7 days.* High, slender, normal villi. Normal pattern. Bacteria (+). 190 $\times$.



Fig. 317. *Fract. irr. 9 Gy $\times$ 2-7 days.* Attack by bacteria and mucinous droplets on the surface. 5000 $\times$.

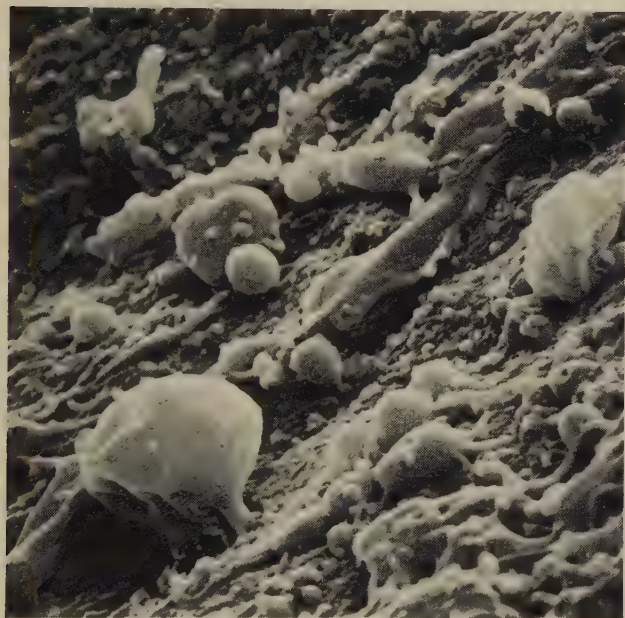


Fig. 318. *Fract. irr. 9 Gy $\times$ 2-7 days.* Peyer's patch. The periphery of a nodule. Cells are penetrating the surface. 5000 $\times$.

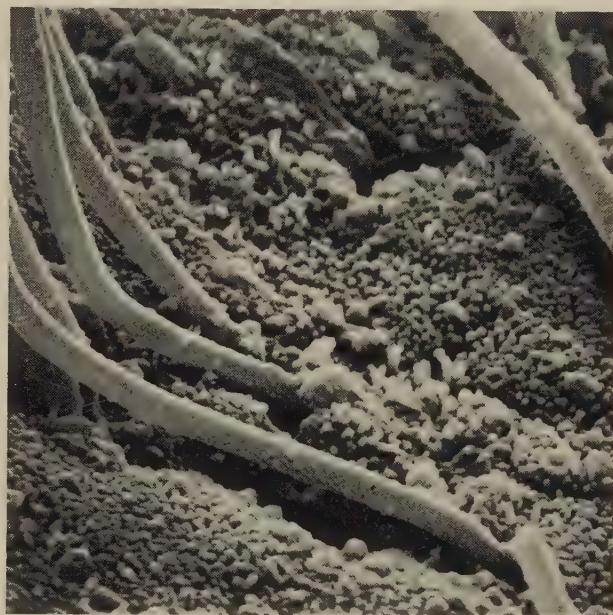


Fig. 319. *Fract. irr. 9 Gy $\times$ 2-7 days.* Peyer's patch. Bacteria in the central section. 5000 $\times$.

Fract. irr. (9 Gy $\times$ 2) - 7 days

At the day when fraction No. 3 should be given, the villi had normalized. They are high, long, slender and leaf-formed with normal extrusion and have rather high Goblet cell activity and attack of bacteria on the upper third-parts. The Peyer's patch has

bacteria in the center and on the peripheral section many penetrating lymphoid cells, which is not seen to that degree in non-irradiated rats. The effect of the first and the second fractions thus seem to have been repaired when the next fraction was to be given.



Fig. 320. Fract. irr. 9 Gy $\times$ 3–3 days. Shortened villi. Massive extrusion on the top. No bacteria. 370 $\times$.

Fract. irr. (9 Gy $\times$ 3) – 3 days

The villi are now shortening with increased, massive extrusion of epithelial cells in spite of already short villi. The single cells on the top have separated from other cells and are ball-formed, but on the side of the villus irregularly formed cells are flat and tightly

pressed against each other. The microvilli are hardly half of normal length on the side of the villus and the cell membrane is visible on the surface. In LM the flat squamous cells can be seen on the side of the villus. No Goblet cells and no bacteria are seen.

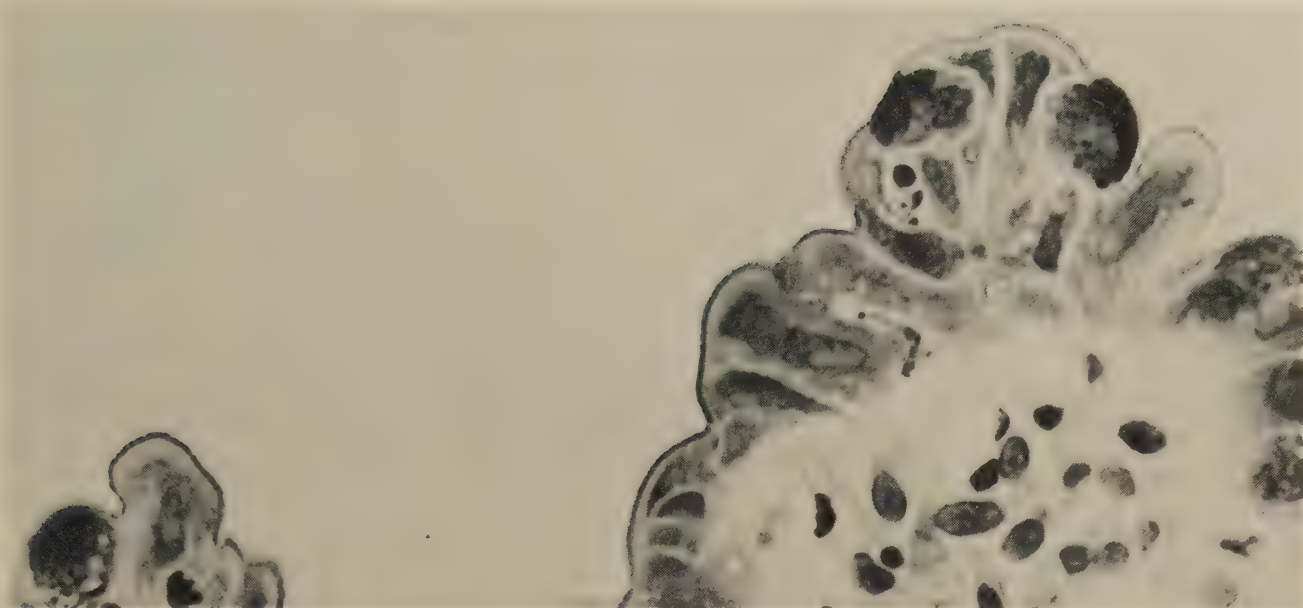


Fig. 321. *Fract. irr. 9 Gy $\times 3$ - 3 days*. Light microscopy picture from the same specimen as in Fig. 322.

Fig. 322. *Fract. irr. 9 Gy $\times 3$ - 3 days*. Enlargement of Fig. 321. Cells in extrusion on the top of every villus. 900 $\times$.





Fig. 323. Fract. irr. 9 Gy $\times$ 3–3 days. Spherical cells. Sparse and short microvilli. Part of a villus with extrusion. 3700 $\times$.

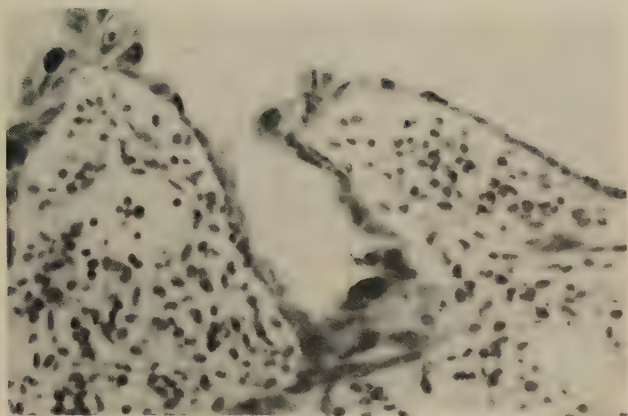


Fig. 324. Fract. irr. 9 Gy $\times$ 3–3 days. LM of the same specimen as in Fig. 323. The cells on the side are now of squamous cell type. Extrusion at the tops.



Fig. 325. Fract. irr. 9 Gy $\times$ 3–3 days. Part of a villus side. Swollen and irregular epithelial cells. No Goblet cells. Short microvilli. 1900 $\times$.



Fig. 326. Fract. irr. 9 Gy $\times$ 3–3 days. Part of a villus side. Tightly packed irregularly formed cells. See also Fig. 324. 1900 $\times$.



Fig. 327. Fract. irr. 9 Gy $\times$ 3 – 3 days. Magnification of Fig. 326. Short sparse microvilli on two of the cell surfaces. 9000 $\times$.

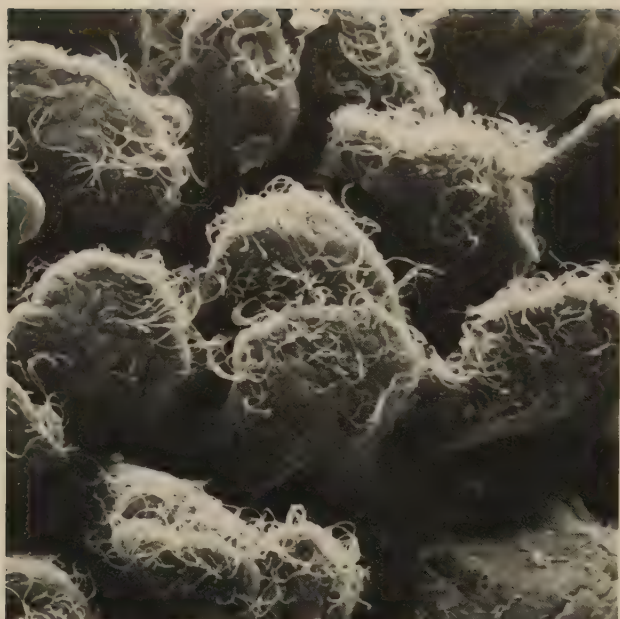


Fig. 328. Fract. irr. 9 Gy $\times$ 3–14 days. High normal villi. A lot of bacteria (+) around the tops. 190 $\times$.



Fig. 329. Fract. irr. 9 Gy $\times$ 3–14 days. Side of a villus. Attack by bacteria and wide, open, emptied Goblet cells. Mucinous droplets on the surface. The cells are not swollen. 1900 $\times$.

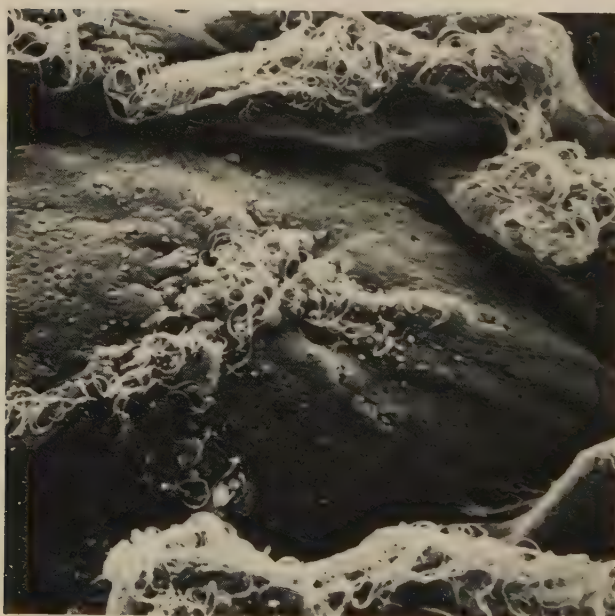


Fig. 330. Fract. irr. 9 Gy $\times$ 3–14 days. One nodule of Peyer's patch. Bacteria in the center and on the surrounding villi. 190 $\times$.

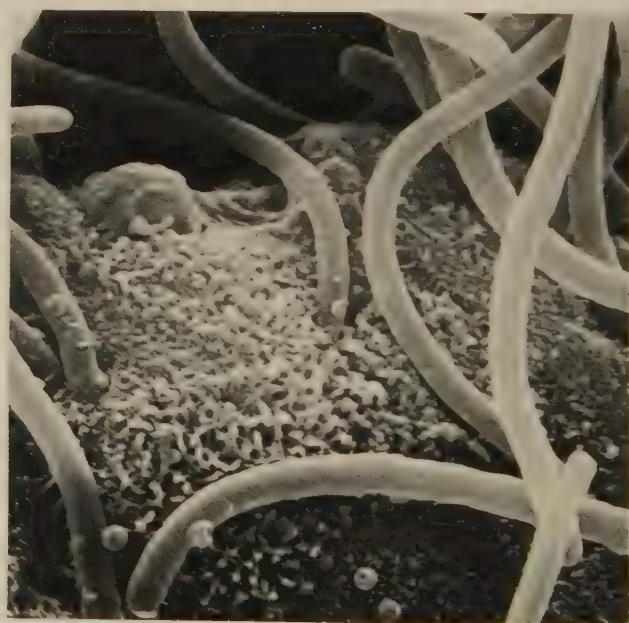


Fig. 331. Fract. irr. 9 Gy $\times$ 3–14 days. High magnification from the center of the Peyer's patch. Intensive activity. Bacteria and sparse microvilli. 4700 $\times$.

Fract. irr. (9 Gy $\times$ 3) – 14 days

Bacteria are attacking both the top and the side and the Peyer's patch as seen at high magnification. The Goblet cells have wide, explosively emptying openings. The microvilli now shape a normal "carpet" on the cells. The SEM-pictures show a hyperactive

villus comparable to the situation 48 hours after 2 Gy or to the initial reaction after high single doses as at 20 GY – 10 min. It is not known whether there is any "over-shooting" in function when the repair is going on.

Weight curves

Weight curves

The weight of the rats has been controlled daily in all fractionation schedules. Figs. 334–339 present the weight curves and reflect the rather slight damage found on SEM. Some other factors may influence the body weight especially during fractionation irradiation, such as fasting, anaesthesia and metronidazole, beside other factors. The radiation sickness and stress may result in fasting of the animals. In Fig. 332 the effect of fasting itself is shown. Five rats were deprived of food, but had water supply, for 6 days and were then given free access to food again. There is a nearly linear decrease in weight down to about 75% followed by an increase to 90% one week later.

As is seen from all weight curves during fractionation irradiation the initial decrease in weight seems to follow the same pattern as in fasting animals. It is repeated at the beginning of each irradiation week, Fig. 335–338, maybe because of a stress factor.

The influence of anaesthesia has been widely discussed, Zauder (1963), Cottier (1966), Devik (1971). The effect on the vascular bed is said to be influencing the effect of irradiation. Devik (1971) did not find any difference between experiments in irradiation of the small intestine with or without anaesthesia. Because all experiments in this study has included

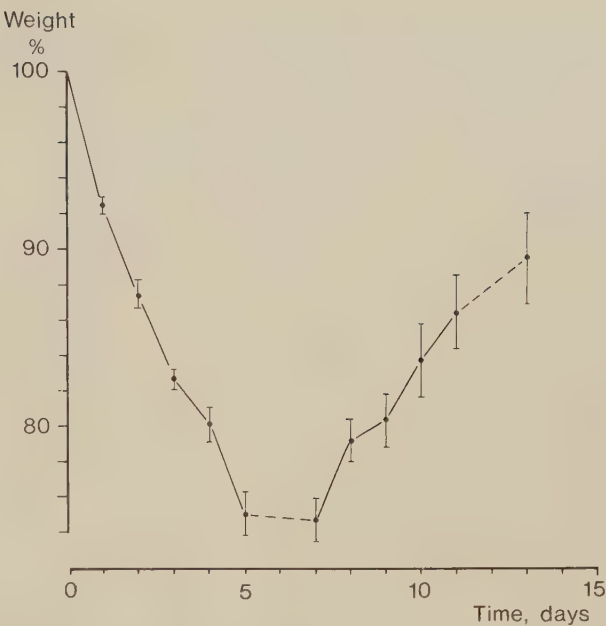


Fig. 332. Body weight. Effect of fasting during 6 days followed by refeeding.

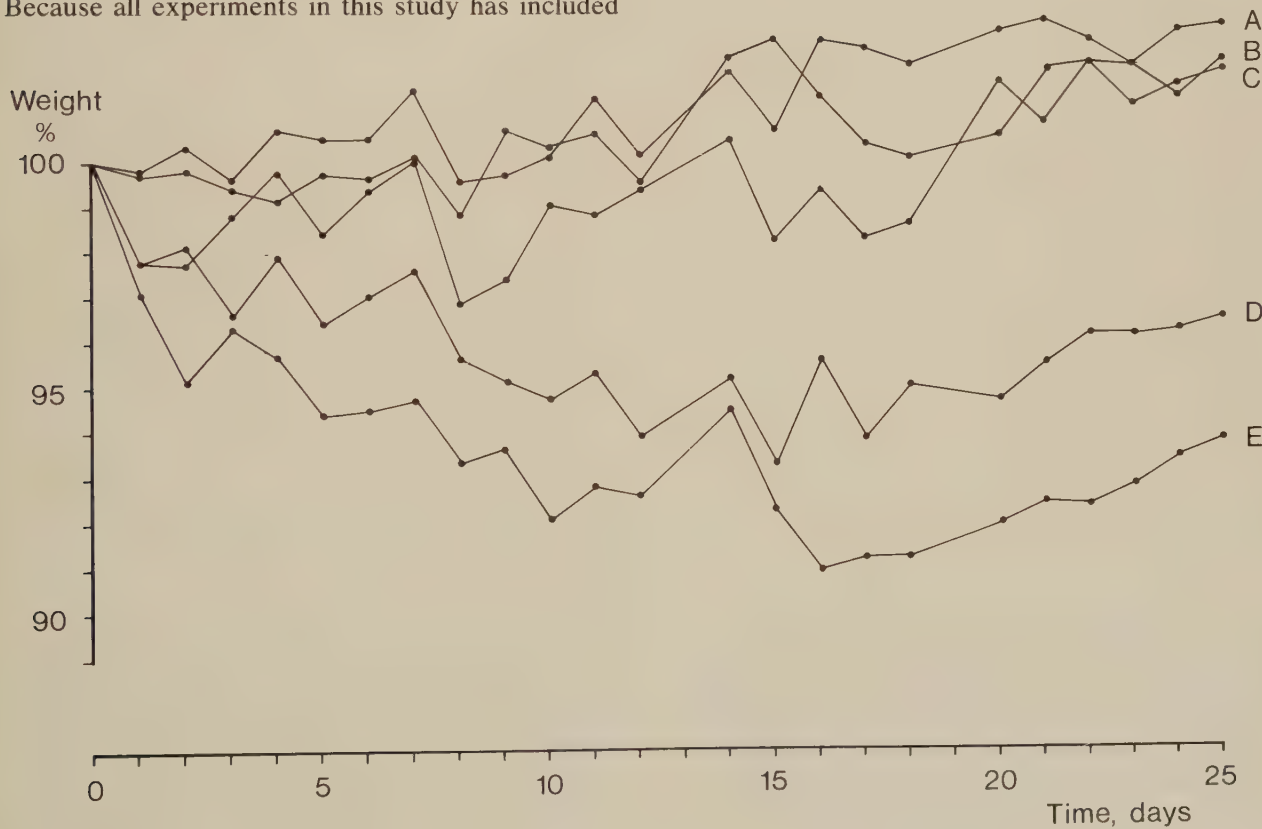


Fig. 333. Body weight. Factors influencing. – A. non fasting animal, control. – B. nembutal^R i.p. once a week. – C. metronidazole, 20 mg, daily. – D. nembutal^R i.p. three times a week. – E. nembutal^R i.p. five times a week.

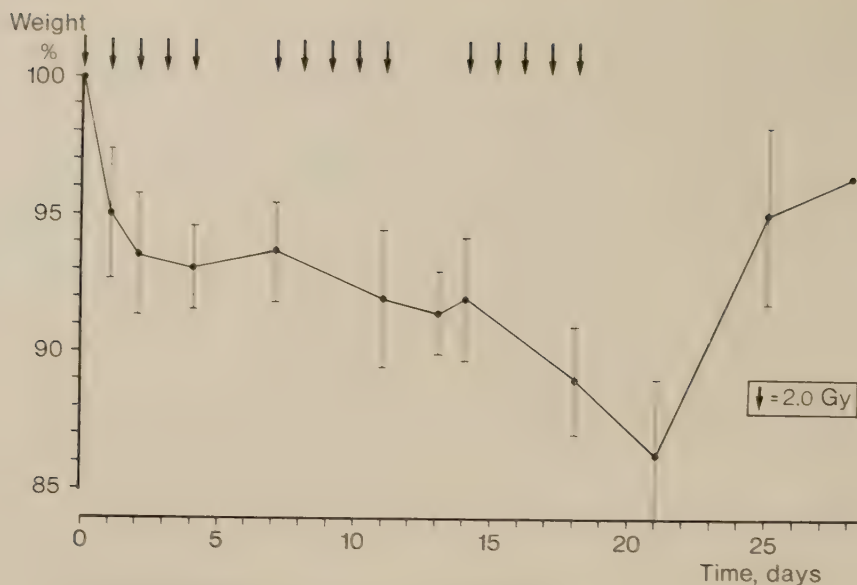


Fig. 334. Body weight. Fract. irr. 5 F (2 Gy $\times$ 5)/week.

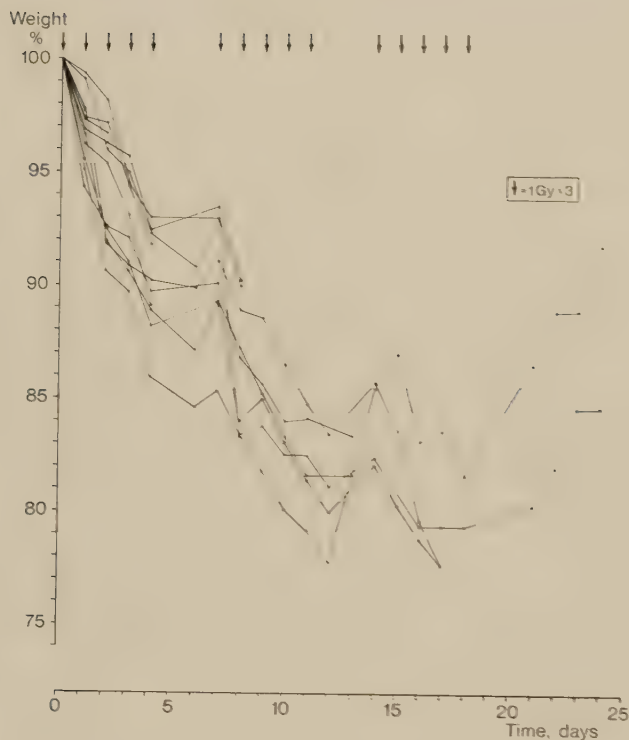


Fig. 335. Body weight. Fract. irr. 15 F (1 Gy $\times$ 3) $\times$ 5/week. Superfractionation.

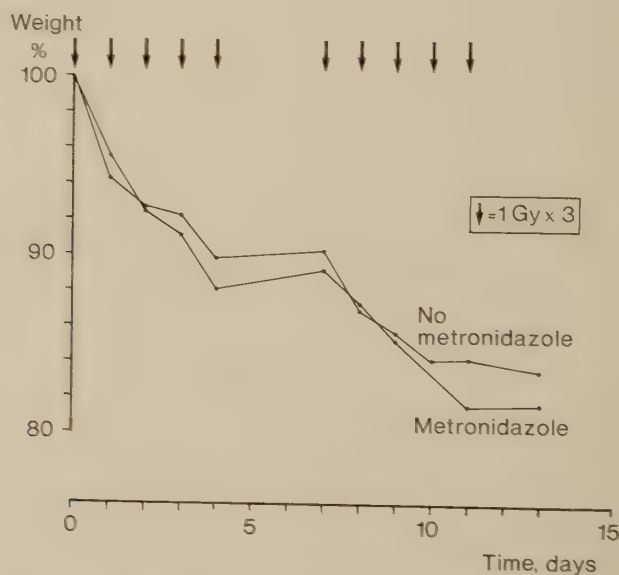


Fig. 336. Body weight. Fract. irr. 15 F (1 Gy $\times$ 3) $\times$ 5/week. Superfractionation. Effect of metronidazole during irradiation.

anaesthesia the results may be comparable. Still in fractionation irradiation with repeated anaesthesia the weight is reduced in relation to the number of fractions. In Fig. 333 different numbers of anaesthesia simulating the fractionation irradiation schedules show the increasing loss of weight and also the increase in weight during the weekends with no anaesthesia and the strong effect of the first anaesthesia in each week. As control, normal non-

anaesthetized animals show a slowly increasing weight. Metronidazole seems not to affect the weight in control animals or in irradiated animals, Fig. 333 and Fig. 336 respectively.

The greatest decrease in weight can be seen in superfractionation, Fig. 335. The SEM pictures do not verify any morphological damage higher than a score number of 1.0 and during the whole irradiation period of three weeks bacteria were attacking the

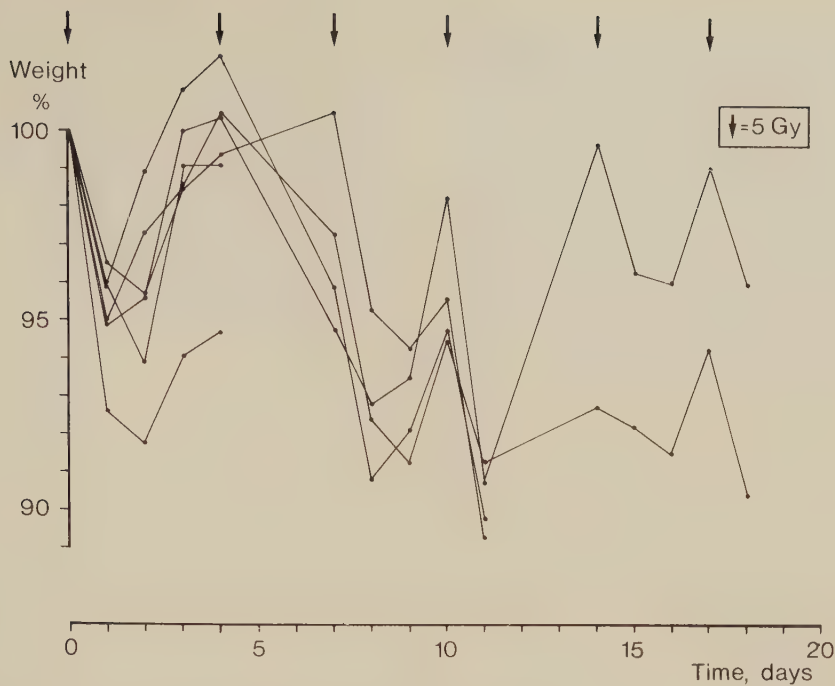


Fig. 337. Body weight. Fract. irr. 2 F (5 Gy $\times$ 2)/week.

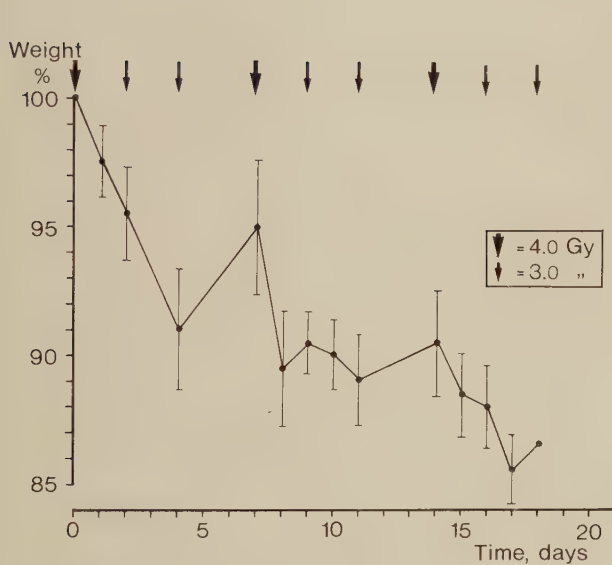


Fig. 338. Body weight. Fract. irr. 3 F (4 + 3 + 3 Gy)/week.

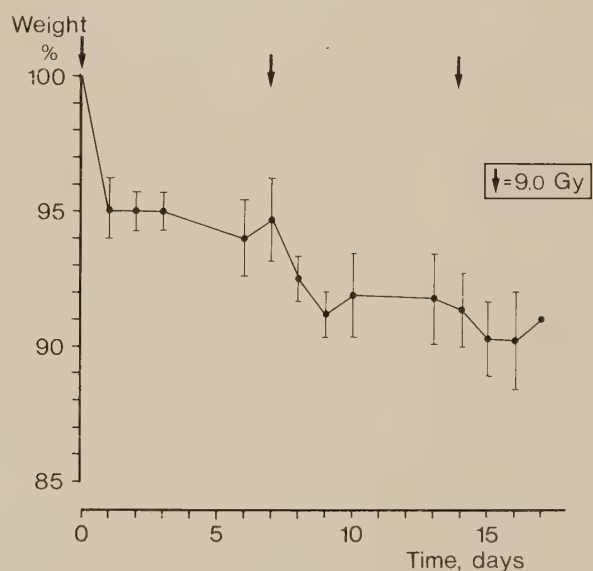


Fig. 339. Body weight. Fract. irr. 1 F (9 Gy)/week.

villi, a sign of non-fasting animal. Thus the anaesthesia in itself may not increase the damage after irradiation when given three times a day but still be a factor influencing the weight. A sharp increase in weight is seen at each weekend and the same phenomenon can be seen in each fractionation regime.

At high fraction size and a few number of fractions, especially 3 F/ week and 2 F/week, the increase in weight is large if the interval between the fractions is more than 72 hours. In shorter intervals the

reduction in weight will continue.

In conclusion the weight of the animal may reflect the damage of irradiation. The schedules of fractionated irradiation used do not give any decrease in weight higher than 10–20% and within this range other factors may have an influence and make the interpretation difficult. The change in weight do anyhow support the SEM results in confirming the slight damage to the intestine even in regimes of 3 F/week and 1 F/week.

Normal sleeping is associated with a parasympathic vagus stimulation from which follows an accumulation of blood in the digestive system. To control if the induced narcosis of the rat was followed by a similar reflex mechanism which could be assumed to influence the result of the experiment, measurement was made by placing a thermistor probe subcutaneously on the chest and another intra-abdominally. The temperature intra-abdominally was measured on 5 rats at $36,5^{\circ}\text{C}$ ($\pm 0,3$) 5 min after

the injection of nembutal and during half an hour the temperature had decreased to $35,8^{\circ}\text{C}$ ($\pm 0,3$). On the chest the temperature at the same time was $36,4$ and $35,4^{\circ}\text{C}$ ($\pm 0,4$) respectively. Contraction of the vascular system of the skin to avoid loss of heat is known to be the usual physiological reaction. The change in the abdominal region was regarded small. Thus, the influence of the parameter of blood-flow could be neglected.

Discussion
Score-curve

Discussion

The advantage of fractionating doses at intervals of about 24 hours and limiting the size of each fraction is a well established fact, and the biological basis for this in the small intestine, as well as in the skin, appears to lie in the great capacity of repair of their epithelial stem cells and to resist sublethal injury, which in fractionated irradiation becomes the predominant process controlling the radiation response. The proliferation must be high enough in the crypts to compensate for the injury given by each fraction. The conventional fractionation regime of 5 F/week and the "super"-fractionation regime of 15 F/week seem to reach a steady state of reaction in 3–4 days and have, according to the score curve in Fig. 340, a nearly constant average reaction around 1.0 until 30 Gy is reached. In these two schedules there is an increased extrusion, which could be explained by an compensation through a high proliferation in the crypts and a shortened generation cycle. (Lesher, 1975). The damage to the crypt is compensated and the villi do not have to stop the extrusion process. Lesher (1967) has also shown that an acute dose does not stop the usual migration of cells from the proliferating compartment to the differentiating compartment and the functional compartment of the villus.

A more intense attack than normal by bacteria and emptying of the Goblet cells is seen on SEM. The interpretation of these findings may assume that the repeatedly given fractions of 2 Gy/day or 1 Gy 3 times a day do not stop the differentiation or the intra-cellular synthesis of epithelial cells. A state of stimulation as seen immediately after high single doses is continuously present during the whole irradiation schedule. Oedema, swollen cells, Goblet cell activity and heavy attack by bacteria even on the extrusion cell surfaces may reflect an increased vascularisation, assuring that no damage to the vascular bed or to the core of the villi is present, that eventual sublethal injury to the crypt cells has been repaired and that a normal function has been established. The difference between fractions of 1.8 Gy and 2.2 Gy was minimal. The attack by bacteria and oedema was lower in the 2.2 Gy/F-regime.

The average reaction for 2 F/week (5 Gy $\times$ 2), is not far from identical with the schedules of 5 F/week, and 15 F/week but the score number, however, is judged a little higher due to the fact that most of the function of the villi has disappeared. Cells in the functional compartment may be deprived of the compound which is attractive for bacteria, since these are not seen. The Goblet cells are closed and migrate up to the top of the villus without being emptied. There is still an oedema. There is almost no

extrusion. The fraction size of 5 Gy is close to the single dose of 6 Gy, which has been given the score of 1.0. The nearly steady state in this fractionation of 5 Gy $\times$ 2 per week may be explained by the rapid increase in surviving ratio, as seen by the crypt counting technique, (Withers and Elkind 1969), at fractionation intervals between 60 and 108 hours, probably resulting from repopulation by survivors after each dose. Thus, the compensating proliferation is enough to avoid an increasing morphologic injury. In high magnification the microvilli are changed and show a sign of "micro" – damage that may explain the absence of bacteria. If metronidazole was given to the rats in the schedule of 5 Gy $\times$ 2/week, the microvilli were more normal and no swelling of the villi was seen, possibly pointing at a kind of protective effect. (Stone and Withers, 1975). Even in this experiment, the bacteria were absent.

Three fractions per week (4 Gy + 3 Gy + 3 Gy) give a score number >1 in the total dose range of 24–30 Gy. Bacteria and Goblet cell activity are not

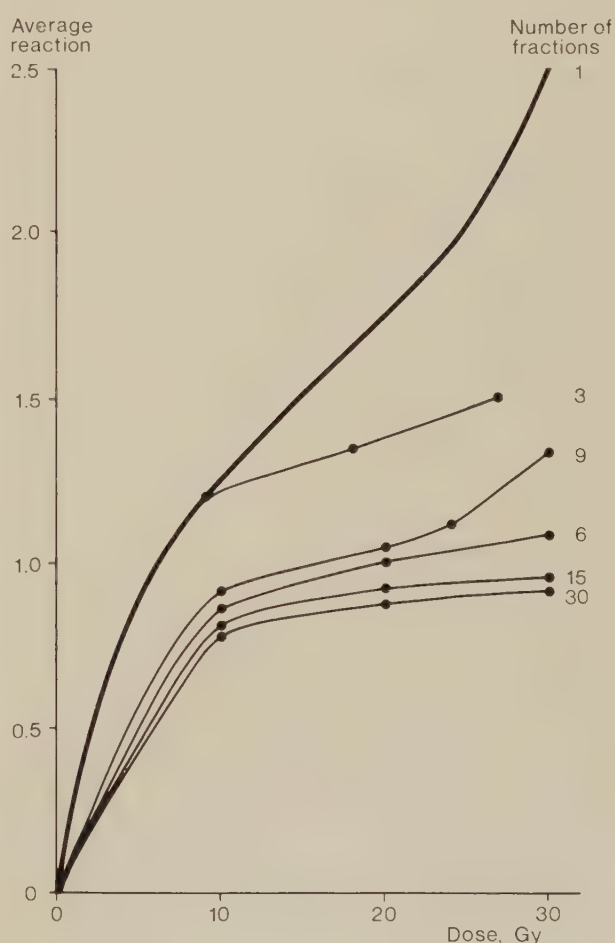


Fig. 340. Average reaction plotted as score numbers in all fractionated irradiation schedules against total dose after one, two and three weeks. The one fraction curve represents the average reaction of different single doses.

seen. Fowler (1965) has shown that a change from 21 F/28 days to 9 F/28 days requires a 20% reduction in the total dose. In this study, the increased damage shown as a high score after the total dose is in agreement with these statements.

Techniques which reduce the capacity of cells for sublethal injury or its repair and which narrow the shoulder of the fractionated dose survival curves are likely to cause a disproportionate increase in lethal injury to the stem cells of the gut, (Withers and Elkind 1969). Decreased survival ratios at fractionation intervals of 46–60 hours have been found consistently. According to van der meer-fiegeen (1973), a maximum effect on the crypt cell number is reached after 48 hours and even if proliferation starts after 24 hours, every fraction in the schedule of 3 F/week will hit the crypt at a critical moment.

The maximum reaction documented by SEM after 9 fractions and 30 Gy in total dose has in this study been given a score number identical to the maximum reaction after 2 fractions of 9 Gy (9 Gy/week) and 18 Gy in total dose. In a log-log diagram with total dose against number of fractions, this will give an iso-effect line with a slope of 0.34, Fig. 341. In a study of the reactions of the jejunum by the crypt counting technique, Withers and Elkind (1970), after multi-fraction-irradiation, a slope of 0.33 was presented, (Withers 1975).

In the small intestine the proliferation rate of the tissue is of such a dignity that any attempt to compare the effect of different doses and fractionation schedules can be misunderstood. Iso-effect curves in the usual way therefore cannot be plotted as for slow proliferating tissue. However, the maximum damage caused by a single dose can be compared with the effect of a fractionated dose giving the same maximum damage. The same comparison can be made between two different fractions. In Fig. 341 total dose versus number of fractions is plotted. 3 fractions of 9 Gy per week, 27 Gy, gives the same maximal reaction as one single dose of 15 Gy, giving a slope of 0.53. Two fractionation schedules have been compared, as mentioned, under the same assumptions. 9 fractions (4 + 3 + 3 Gy)/week with a total dose of 30 Gy and 2 fractions, 9 Gy/week, with a total dose of 18 Gy give the same score and a slope of 0.34. Finally 21 fractions in the superfractionation schedule (1 Gy × 3) × 7 with a total dose of 21 Gy the same score as a single dose of 6 Gy and a slope of 0.41. The difference between the slopes is difficult to explain. Many parameters are involved, damage and repopulation of the crypts, effect on the vascular bed and the stroma below the surface and SEM gives the reaction of the mucosal surface alone but at the same time a total expression of the end result of all factors.

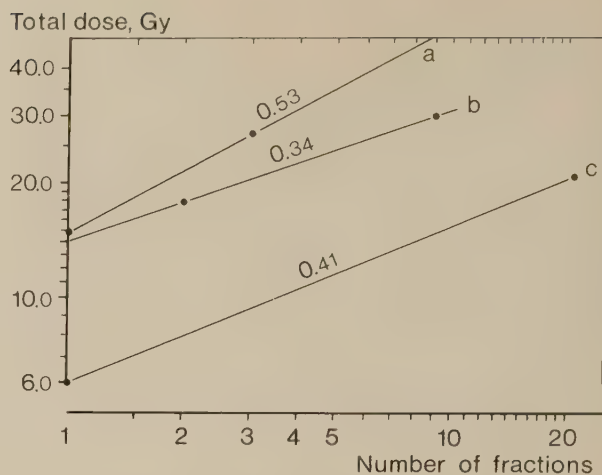


Fig. 341. Comparable effects plotted in a log-log diagram with total dose against the number of fractions.

1 F/week, 9 Gy, gives, as seen in Fig. 340, a still higher score number. One fraction, 9 Gy, is the single dose which represents the dose level where the bacteria disappear, and the dose where the injury to the crypt seems to be large enough to cause the villus to start a shortening process by extruding clusters of cells from the top (Fig. 108). At a single dose of 9 Gy, the injury is nearly repaired 7 days after irradiation. On this day, the next fraction is given. Again, bacteria disappear, but extrusion is not seen. The villi are finger-like and the epithelial cells have irregular form. The third fraction of 9 Gy gives a great extrusion with a reduction in the height of the villi. No bacteria or Goblet cells are seen. The maximum average score number is estimated to 1.5, equal to the score given to the maximum reaction after 15 Gy single dose. In both cases full repair can be documented. 15 Gy single dose was found to be some kind of a “crossroad” between life and death for the animals.

A single dose of 15 Gy gives the same score as three fractions of 9 Gy per week = 27 Gy. The necessary increase in dose is 80%. 18 Gy in two fractions give the same score as 30 Gy given in nine fractions, requiring an additional 12 Gy or 66% increase in total dose.

The opposite kind of the fractionation schedule to 1 F/week, 15 F/week, “super”-fractionation, resulted in a nearly unchanged state not only during 2 weeks and up to 30 Gy, but also for the third week and a total dose of 45 Gy. This result confirms the theory by Dutrieux *et al.* (1973) that no further dose is necessary in schedules with more than 20 fractions and fractions lower than 3 Gy to attain the same effect. In the super-fractionation regime, a lot of bacteria were attacking the villi all the time. When

metronidazole was given to the animals, no bacteria could be detected and the SEM-pictures could almost be compared to the condition of fasting animals. The conclusion was that a slightly protective effect had been established. However, a minor degree of toxic effect could not be denied. *Denekamp et al.* (1974) in skin studies, and *Stone and Withers* (1975) in small intestine studies, have demonstrated a kind of protective effect of metronidazole.

In the treatment of carcinoma of the *cervix uteri* in advanced stages, metronidazole could be given as a sensitizer in order to increase the irradiation effect in the hypoxic parts. At the same time, an optimal fractionation schedule is desirable. Reactions in the small intestine can not be avoided and the hypothesis that the anaerobe bacteria disappear when metronidazole is used during irradiation in order to reduce symptoms such as diarrhoea, may be wrong.

This study by SEM supports the view that the bacteria are living in symbiosis with the functional epithelial cells. They are not penetrating the cells in fasting animals and have disappeared after high single doses, >6 Gy, and after fractionation schedules giving morphological injury of the same degree. Advanced reactions or late stages of injury do not show any sign of bacteria present on the mucosa. However, metronidazole may still have effects on anaerobe bacteria and protect an injured mucosa.

Fasting animals irradiated with $2.2 \text{ Gy} \times 5$ do not show bacteria and the Goblet cells do not empty their contents. Non-fasting animals irradiated in the same way show a "stimulation" with massive attack by bacteria and Goblet cells emptying their contents explosively. The condition of fasting was a stronger

factor than irradiation. This may be in accordance with the results of hypoxia and irradiation as reported by *Larsson and Sténson* (1965) and *Penn et al.* (1975).

The curves of body weight change during the different irradiation regimes show a rapid decrease in weight during the first days. There is a regain especially when the interval between each fraction is long, and also at every weekend, when no irradiation is given. With increasing number of fractions during which anaesthesia is given, the reduction in weight increases. Anaesthesia may be responsible for part of this decrease. On the other hand 5 F/week and 15 F/week show a lot of bacteria all the time, confirming that the animals are non-fasting.

A report by *Carr et al.* (1979) is the only SEM-study on the effects of fractionated irradiation of the intestinal mucosa. A comparison was made between SEM-documented effects and the crypt-counting technique in three different fractionation schedules. Their conclusion was a lack of correlation between the SEM and the crypt-counting method, but they claimed that SEM is valuable in judging the effect of irradiation on the surface, which has also been proven in this study.

In conclusion, this fractionated irradiation study shows that the SEM-technique gives a detailed document of the reactions of the small intestinal mucosa, which makes it possible to compare different fractionation regimes. When the three-dimensional information about the morphology and about function are taken into consideration, there seems to exist a correlation between the reaction of the surface and the well known effects of the crypts and of the body weight.

Chapter IX

Summary

SEM has opened a new view in examining the surface of organs through its third-dimensional picture and its high resolution power. A lot of pathologic conditions could be studied and better understood. Irradiation effect on the small intestine is well-known with the essential work done on the crypts of Lieberkühn. Because of the rather sophisticated mechanisms at the surface of the small intestine a SEM-study was considered worthwhile since the effect of irradiation of the abdomen in the clinic is directly dependent on the reaction of the intestine.

The aim was to simulate the treatment of pelvic tumors in human beings and to simulate the conventional and not-conventional schedules of fractionated irradiation.

This SEM-study of the small intestine reaction was performed as a partial abdominal irradiation during standardized conditions, using a model, constructed for the rat and using 137 -caesium irradiation technique.

In SEM-preparation of the specimens a special technique of dehydration had to be worked out because of the oedema after irradiation. After a control with air-drying and critical-point technique with no difference in results, the air drying method was continuously used.

At first the normal intestine, the ileum, was studied. A quite different picture was seen in fasting animals as compared to non-fasting animals. Fasting animals showed high and slender villi with a moderate extrusion of epithelial cells on the ridge of the tops. No bacteria were seen and the Goblet cells migrated along the side of the villus. In the non-fasting animals, the villi were retracted with advanced folding on the sides and a massive attack by bacteria at the upper thirds was seen as well as mucinous granulae spread over the epithelial cell surfaces. By SEM it could be characterized as a state of passivity against a state of activity.

The effect of single dose-irradiation was recorded in order to get a reference material, used in comparison to other experimental works with the 2-dimensional technique of LM and TEM. Later on the

results of fractionated irradiation could be correlated to these findings. Ten different single doses were given (2, 3, 6, 9, 12, 15, 20, 22.5, 25 and 30 Gy) and the reaction was studied at ten different intervals at each dose. The effect was examined at 5–60 minutes after 20, 25 and 30 Gy, at 2–7 days after all doses and at 14 and 21 days after 15, 20 and 22.5 Gy. The irradiation effect was evaluated using a score system on an arbitrary scale from 0.0 to 4.0 and the average reactions were plotted as effect curves.

The initial effect after single doses of 20–30 Gy was a heavy attack by bacteria at the tops of the villi and at the centers of the Peyer's patches and an explosively emptying of the Goblet cells. Lymphoid cells were seen penetrating the so called M-cells at the peripheral part of the Peyer's patches. The villi were swollen. The bacteria vanished after about 30 minutes. After a few hours the villi could be compared to the villi in a fasting condition.

There was almost no morphological damage seen during the first 48 hours following the different single doses given. Low doses initiated the same picture as described. The score effect curves showed a maximum reaction after 72 hours at doses up to 15 Gy. A week later, full repair was seen after doses of 6–12 Gy. The reaction after 15 Gy was unchanged from day 3 to day 7. Doses higher than 15 Gy resulted in increasing damage at day 5–7, resulting in disappearance of the villi and a plain surface with areas of ulceration. Full repair was not complete at day 21 after 20, 22.5 and 25 Gy. Most of the rats given 25 and 30 Gy died between day 7 to 10. The $LD_{50(30)}$ in this partial abdominal irradiation was 22.5 Gy. Weight curves and score effect curves showed that the single dose of 15 Gy was a crossroad.

The reaction of the single doses could be divided in different levels:

A. A single dose of about 6 Gy results in a maximum damage to the crypt cells after 48 hours while the villi as seen on SEM have a triangular or pyramidal form after 48 to 72 hours. There are still bacteria on the tops and no increased extrusion of cells. This is the maximum reaction of 6 Gy. Proliferation in the

crypts will probably send new cells migrating up to the villus before the villus has to react to the lack of cells by extruding more cells at the top and shorten in height.

B. 9 Gy shows an increased reaction. Now the bacteria have disappeared and clusters of cells leave the tops of the villi. A lag period of 48 hours exists before the villus reacts by loosing cells and retract (the normal turn-over time is 36–48 hours). After 9 Gy the repair is finished within 7 days.

C. Another level is seen after the 15 Gy-dose where a kind of steady state exists from day 3 to day 14. The height of the villi is reduced to 50% and cells are loosening from the tops. Repair can be seen at day 14 but is not finished at day 21. Goblet cells and bacteria are not seen.

D. Five days after 20 Gy and 22.5 Gy, the villi have disappeared completely. The flat surface of the mucosa is partly denudated. As seen from the weight curve there exists a critical period between day 12 and 20, during which the rat may die or recover. If the mucosa is denudated to a degree below 50% there seems to be a chance of survival. Still, with no further decrease in weight and with restored mucosal covering, the rat may die during the mentioned period. Repair is seen as irregularly growing villi of different size. Until full height of the villi is reached, full function does not seem to be regained. A sign is the attack by the bacteria.

In different fractionation schedules, 5 F/week, 2 F/week, 3 F/week, 15 F/week and 1 F/week, the same score system was used in judging the reaction at every 24 hours during the overall time of about three weeks. The conventional 5 F/week given as 2 Gy/day resulted in a steady state from a total dose of 10 Gy to a total dose of 30 Gy. A score number not higher than 1.0 was recorded. The same range of reaction after both 2 F/week ($5 \text{ Gy} \times 2$) and 15 F/week (superfractionation $1 \text{ Gy} \times 3/\text{day}$) was recorded during the fractionated irradiation. 3 F/week given as $4 \text{ Gy} + 3 \text{ Gy} + 3 \text{ Gy}$ per week at 48 hours interval did however give an increasing score number from about a total dose of 20 Gy to 30 Gy. The maximum damage to the crypts is reached after 48 hours. At this moment the next fraction is given which may explain the higher score in this schedule. 1 F/week given as 9 Gy and three times results in a still higher score, comparable to the

effect of a single dose of 15 Gy. The score effect curve may confirm the known fact that 3 F/week will require reduction in the total dose in order to give the same effect. Both 3 F/week and the schedule of 1 F/week do not give any serious damage to the intestinal mucosa, as seen by SEM. The maximum effect is a reduction of the height of the villi to 50% and an increased extrusion just as seen after a single dose of 15 Gy. The difference between 5 F/week, 15 F/week and 2 F/week seems to be caused by disturbances in the function. 2 F/week result in a disappearance of bacteria and in closed Goblet cells. From the steady state of 2 F/week, 5 F/week and 15 F/week with low score number, it is assumed that these fractionation schedules can be used, though they are not the optimum of fractionation of the small intestine. 2 F/week with an interval of 72–96 hours do not give any serious damage in this highly proliferative tissue.

Metronidazole was given in the superfractionation and the conventional schedules. It seems as if the fusiform bacteria disappear and that the surface of the intestine can be given a lower score. The effect of metronidazole may be bactericid or/and to have a toxic effect to the microvilli, which secrete the substance that may attract the fusiform bacteria in the normal intestine. The reason behind clinical symptoms as diarrhoea is difficult to deduce from the SEM-pictures. 1 F/week and 3 F/week, which have been given the highest score, do not result in any denudation nor 2 F/week or $2.2 \text{ Gy} \times 5/\text{week}$ give any morphological explanation. Thus these symptoms may be a result of functional disturbances.

Peyer's patches, localized in the lower ileum, are damaged by the irradiation. Following high single doses or high fractions in fractionated irradiation the nodules of the Peyer's patches shrink and disappear between the villi. At different doses leakage of numbers of lymphoid cells can be followed through narrow openings in the membrane of the M-cell. No penetration of lymphoid cells has been recorded through the epithelial surface of the villi.

In conclusion SEM is able to demonstrate not only morphological changes in detail of the mucosa but also functional disturbances. Thus there exist a close correlation between the SEM-picture of the mucosa of the small intestine and the weight of irradiated animals.

The degree of magnification is specified in all legends.

In Table 3 the real distance of 10 mm on the SEM-picture at the different magnifications used are presented.

Table 3. *Transformation of 10 mm to the real distance.*

Magnification	10 mm corresponds to
120 ×	83 μm
240 ×	42
480 ×	21
600 ×	17
1,200 ×	8
2,400 ×	4
5,200 ×	2
6,000 ×	1.7
12,000 ×	0.8
24,000 ×	0.4
48,000 ×	0.2

References

- Abrams G. D., Bauer H., Sprintz H. Influence of the normal flora on mucosal morphology and cellular renewal in the ileum. A comparison of germ-free and conventional mice. *Lab Invest* 12:355, 1963.
- Adinolfi M., Glynn A. A., Lindsay M., Milne C. M. Serological properties of Gama-A-antibodies to *Escherichia coli* present in human colostrum. *Immunology* 10:517, 1966.
- Alm P., Fernö M., Friberg L.-G., Norgren A. Estradiol and progesterone receptor concentration in endometrial carcinoma related to histological parameters and ionizing irradiation. *Cancer Treatment Report* 63, no 7, 1189, 1979.
- Anderson J. A., Taylor A. B. Scanning and Transmission Electron Microscopic Studies of Jejunal Microvilli of the Rat, Hamster and Dog. *J Morph* 14:281, 1973.
- Anderson J. H., Withers R. H. Scanning electron microscope studies of irradiated rat intestinal mucosa. *Scanning Electron Microscopy/1973 (Part III)*. Proceedings of the workshop on Scanning Electron Microscopy in Pathology III Research Institute Chicago, Illinois 60616, USA.
- Anderson T. F. Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope. *Trans NY Acad Sci* 13:130, 1951.
- Arborth B., Bell P., Brunk U. and Collins V. P. The osmotic effect of Glutaraldehyde during fixation. A Transmission Electron Microscopy, Scanning Electron Microscopy and Cytochemical Study. *J Ultrastructure Res* 56:339, 1976.
- Ashworth C. T., Luibel F. J., Stewart S. C. The fine structural localization of adenosine triphosphatase in the small intestine, kidney and liver of the rat. *The J Cell Biology* 17:1 1963.
- Balcerzak S. P., LANE W. C., Bullard J. W. Surface structure of intestinal epithelium. *Gastroenterology* 58:49, 1969.
- Becciolini A., Ravina A. Effect of ionizing radiation on intestinal disaccharidases in rats. *Brit J Radiol* 43:150, 1970.
- Becciolini A., Cariaggi P., Arganini L., Castagnoli P., de Giuli P. Effect of 200, 650 and 1200 R on the intestinal disaccharidases and dipeptidases. *Acta Radiol Ther Phys Biol* 13:141, 1974.
- Bellamy J. E., Nielsen N. O. Immune-mediated emigration of neutrophils into the lumen of the small intestine. *Infection Immunity* 9:615, 1974.
- Berry R. J., Wiernik C., Patterson T. J. S. Skin tolerance to fractionated X-irradiation in the pig-how good a predictor is the NSD-formula? *Brit J Radiol* 47:185, 1974.
- Boyde A., Tresman R. L., Willis R. A. Outgrowth from chick embryo spinal cord in vitro studies with the scanning electron microscope. *Z Zellforsch Mikrosk Anat* 90:1, 1968.
- Brown A. L. Microvilli of the human jejunal epithelial cells. *The J Cell Biology* 12:623, 1962.
- Cairnie A. B., Lamerton L. F., Steel G. G. Cell proliferation studies in the intestinal epithelium of the rat. *Exp Cell Res* 39:539, 1965.
- Carr K. E., Toner P. G. Scanning electron microscopy of rat intestinal villi. *Lancet* 570, 1968.
- Carr K. E., Toner P. G. Surface studies of acute radiation injury in the mouse intestine. *Virchows Arch Abt B Zellpath* 11:201, 1972.
- Carr K. E., Dunn J. S., Toner P. G. Scanning electron microscopy of the alimentary tract. *Scot med J* 19:211, 1974.
- Carr K. E., Hamlet R., Nias A. H. W., Watt C. Lack of correlation between villus and crypt damage in irradiated mouse intestine. *Brit J Radiol* 52:485, 1979.
- Cassady J. R., Order S., Camitta B., Marck A. Modification of gastrointestinal symptoms following irradiation by low dose rate technique. *Int J Radiat Oncol* 1:15, 1975.
- Clark J. M., Glagov S. Evaluation and publication of scanning electron micrographs. *Science* vol 192:4241, 1360, 1976.
- Cohen A. L., Marlow D. P., Garner G. E. A rapid critical point method using fluorocarbons (Freons) as intermediate and transitional fluids. *J Microscopie* 7:331, 1968.
- Conard R. A. Some effect of ionizing radiation on the physiology of gastrointestinal tract: a review. *Radiation Res* 5:167, 1956.
- Cottier H. Histopathologie der Wirkung ionisierender Strahlen auf höhere organisme (Tier und Mensch). *Handbuch der medizinischen Radiologie* II/2:64, Springer Verlag, Berlin-New-York 1966.
- Cronkite E. P., Bond V. P. Radiation Injury in Man, Its Chemical and Biological Basis, Pathogenesis and Therapy. Charles C. Thomas, Springfield, Ill., 1960.
- Demling L., Becker V., Classen M. Examination of the mucosa of the small intestine with the scanning electron microscope. *Digestion* 2:51, 1969.
- Dencker H., Johnsson J.-E., Liedberg G. Surgical aspects of radiation injury to the small and large intestines. *Acta*

- Chir Scand 137:692, 1971.
- Denekamp J., Michael B. D., Harris S. R. Hypoxic cell radiosensitizers: Comparative tests of some electron - affinic compounds using epidermal cell survival in vivo. *Radiat Res* 60:119, 1974.
- Deveney C. W., Lewis F. R., Schrock T. R. Surgical management of radiation injury of the small and large intestine. *Dis Col & Ract* 19:25, 1976.
- Devik F. Intestinal cell kinetics in irradiated mice. A quantitative investigation of the acute reaction to whole body roentgen irradiation. *Acta Radiol Ther Phys Biol* 10:129, 1971.
- Doran G. A., Heydon R. Scanning electron microscopy of developing villi in rat small intestine. *J Anat* 110:507, 1971.
- Drees D. T., Waxler G. L. Enteric colibacillosis in gnotobiotic swine. A fluorescence microscopic study. *AM J Vet Res* 31:1147, 1970.
- Drucker D. E., Whittaker D. K. Examination of certain bacterial colonies by scanning electron microscopy. *Microbios* 4(14):109, 1971
- Dubos R. J., Schaedler R. W., Stephens M. The effect of antibacterial drugs on the fecal flora of mice. *J Exp Med* 117:231, 1963.
- Dutriex J., Wambersie A., Bounik C. Cellular recovery in human skin reactions: Application to dose fraction number overall time relationship in radiotherapy. *Eur J Cancer* 9:159, 1973.
- Erlandsen S. L., Chase D. G. Morphological alterations in the microvillous border of villous epithelial cells produced by intestinal microorganisms. *Am J Clin Nutr* 27:1277, 1974.
- Fabrikant J. I. *Radiobiology Year Book Medical Publishers*, 1972.
- Fowler J., Stern B. E. Dose-rate effects: Some theoretical and practical considerations. *Brit J Radiol* 33:389, 1960.
- Fowler J. F. The estimation of total dose for different numbers of fraction in radiotherapy. *Brit J Radiol* 38:365, 1965.
- Frankendahl B. Experimentella och kliniska studier av strålreaktioner i mag-tarmkanalen. Umeå univ. Med. Diss. 5, 1973.
- Friedman N. B. Cellular dynamics in the intestinal mucosa: the effect of irradiation in epithelial maturation and migration. *J Exp. Med.* 81:553, 1945.
- Friberg L-G. SEM-studies on the irradiation effect of the small intestine. Third Congress of Radiology, Edinburgh, 1975.
- Friberg L-G. Early effects of single dose and fractionated irradiation of the small intestine of the rat studied by SEM. Digest of the Ninth Nordic Meeting on Clinical Physics, 1977.
- Fujii R. Quantitation of the number of villi and crypts in the intestine of rodents animals. *Experimentia* 28:10, 1209, 1972.
- Goldberg D. M. Changes in intestinal and renal function after pelvic irradiation: correlation of clinical and experimental observations. *Clin Radiol* 23:225, 1972.
- Green N., Iba G., Smith W. R. Measures to minimize small intestine injury in the irradiated pelvis. *Cancer* 35:1633, 1975.
- Greenberger N. J., Isselbacher K. J. Malabsorption following radiation injury to the gastrointestinal tract. *Am J Med* 36:450, 1964.
- Greenberger N. J. The intestinal brush border as a digestive and absorptive surface. *Am J Med SCI* 258:144, 1969.
- Hagemann R. F., Sigdestad C. P., Leshner S. Intestinal crypt survival and total and per crypt levels of proliferative cellularity following irradiation: fractionated X-ray exposures. *Radiat Res* 47:149, 1971.
- Hagemann R. F., Sigdestad C. P., Leshner S. Intestinal crypt survival and total and per crypt levels of proliferative cellularity following irradiation: single X-ray exposures. *Radiat Res* 46:533, 1971.
- Hamlet R., Carr K. E., Toner P. G., Nias A. H. W. Scanning electron microscopy of mouse intestinal mucosa after cobalt 60 and D-T neutron irradiation. *Brit J Radiol* 49:624, 1976.
- Hampton J. C., Rosario B. The attachment of microorganisms to epithelial cells in the distal ileum of the mouse. *Lab Invest* 14:1464, 1965.
- Hampton J. C. A comparison of the effects of X-irradiation and colchicine on the intestinal mucosa of the mouse. *Radiat Res* 28:37, 1966.
- Hewitt H. B., Wilson C. W. A survival curve for mammalian cells irradiated in vivo. *Nature (London)* 183:1060, 1959.
- Hodges G. M. A scanning electron microscope study of cell surface and cell contacts of spontaneously transformed cells in vitro. *Eur J Cancer* 6:235, 1970
- Huges K. Recent knowledge of the strict anaerobes of the gut. *Aust Veterin J* 48:508, 1972.
- Hopwood D. Some aspects of fixation with Glutaraldehyd. *J Anat* 101:83, 1967.
- Imondi A. R., Balis M. E., Lipkin M. Changes in enzyme levels accompanying differentiation of intestinal epithelium cells. *Exp Cell Res* 58:323, 1969.
- Ishikawa H., Bischoff R., Holtzer H. Formation of arrow-head complexes with heavy meromyosin in a variety of cell types. *J cell Biol* 43:312, 1969.
- Ito S. The enteric surface coat on cat intestinal microvilli. *J Cell Biol* 27:475, 1965.
- Joelsson I., Räf L., Söderberg G. Stenosis of the small bowel as a complication in radiation therapy of carcinoma of the uterine cervix. *Acta Radiol Ther Phys Biol* 10:593, 1971.
- Jolles B., Remington M., Simon-Reuss I. Indirect radiation effects and diffusible factors in irradiated tissues (stromatex). *Acta Radiol Stockh.* 56:57, 1961.
- Klainer A. S., Perkins R. L. Normal and abnormal morphology of microorganisms. A scanning-beam electron microscope study. *JAMA*, 215, 10:1655, 1971.
- Lajtha L. G., Oliver R. Cell population kinetics following different regimes of irradiation. *Brit J Radiol* 35:131, 1962.
- Larsson B., Sténson S. Reduction of radiation damage to the intestinal mucous membrane by local hypoxia. *Nature* 205:364, 1965.
- Lea D. E. A. *Actions of Radiations on living Cells*. 2nd ed. Cambridge, England, Cambridge University Press, 1956.

- Leblond C. P., Stevens C. E. The constant renewal of the intestinal epithelium in the albino rat. *Anat Rec* 100:357, 1948.
- Lee A., Gordon J., Dubos R. J. Enumeration of the oxygen-sensitive bacteria usually present in the intestine of healthy mice. *Nature (London)* 220:1137, 1968.
- Leshner S. Compensatory reactions in intestinal crypt cells after 300 roentgens of cobalt-60 gamma irradiation. *Radiat Res* 32:510, 1967.
- Leshner S., Bauman J. Recovery of reproductive activity and the maintenance of structural integrity in the intestinal epithelium of the mouse after single dose whole-body ^{60}Co X-ray exposures. In: *Effects of radiation on cellular proliferation and differentiation*, p. 507. International Atomic Energy Agency, Vienna, 1968.
- Leshner S., Bauman J. Cell kinetics in intestinal epithelium. *Nat Ca Instit Monogr* 30:186, 1969.
- Leshner J., Leshner S. Effects of Single-Dose, Whole-Body, ^{60}Co Gamma Irradiation on Number of Cells in DNA Synthesis and Mitosis in the Mouse Duodenal Epithelium. *Radiat Res* 43:429, 1970.
- Leshner S., Cooper J., Hagemann R., Leshner J. Proliferative patterns in the mouse jejunal epithelium after fractionated abdominal X-irradiation. *Radiat Res Quarterly* 10:229, 1975.
- Ludwig H., Brück H. J., Metzger H. Kombinierte lichtmikroskopische Untersuchungen als Vorbereitung zur Rasterelektronenmikroskopie unter besonderer Berücksichtigung der Morphologie des Fibrins. *Leitz Mitt. Wiss. u. Techn. Bd. V Nr 7*:207, 1972.
- Luse S. A. Preparation of biologic specimens for scanning electron microscopy. *Stereoscan* 70:149, 1970.
- McGulloch E. A., Till J. E. The sensitivity of cells from normal mouse bone marrow to gamma radiation in vitro and in vivo. *Radiat Res* 16:822, 1962.
- Marcial V. A., Bosch A. Fractionation in Radiation Therapy of Carcinoma of the Uterine Cervix: Results of Prospective Study of 3 VS. 5 Fractions per Week. *Front Radiation Ther Onc*, Vol 3:238, 1968.
- Marsh M. N., Swift J. A. A study of small intestine mucosa using the scanning electron microscopy. *Gut* 10:940, 1969.
- Maruyama Y., van Nagell J. R., Utley J., vider M. L., Parker J. C. Radiation and small bowel complications in cervical carcinoma therapy. *Radiology* 112:699, 1974.
- Mason G. R., Dietrich P., Friedland G. W., Hanks G. E. The radiological findings in radiation - induced enteritis and colitis. A review of 30 cases. *Clin Radiol* 21:232, 1970.
- Matuzawa T., Wilson R. The intestinal mucosa of Germfree mice after whole-body X-irradiation with 3 kiloroentgens. *Radiat Res* 25:15, 1965.
- Meller S. M., Coppe M. R., Ito S., Waterman R. E. Transmission electron microscopy of critical point dried tissue after observation in the scanning electron microscope. *Anat Rec* 176:245, 1973.
- Merrell B. R., Walker B. R. I., Gillmore J. D., Porvaznik M. Scanning electron microscopy observations of the effects of hyperbaric stress on the populations of segmented filamentous intestinal flora on normal mice. *Scanning electron microscopy (1979) III*. SEM Inc AMF O'Hare IL 60666, USA.
- Moog F., Grey R. D. Alkaline phosphatase isozymes in the duodenum of the mouse: Attainment of pattern of spatial distribution in normal development and under influence of cortison or actinomycin D. *Development Biology* 18:481, 1968.
- Nelson D. P., Mata L. J. Bacterial flora associated with the human gastrointestinal mucosa. *Gastroenterology* 58:156, 1970.
- Newman A., Katsaris J., Blendis L. M., Charlesworth M., Walter L. H. Small intestinal injury in women who have had pelvic radiotherapy. *Lancet*, 7844:1471, 1973.
- Nordström C., Dahlqvist A., Josefsson L. Quantitative determination of enzymes in different parts of the villi and crypts of rat small intestine. Comparison of alkaline phosphatase disaccharidase and dipeptidases. *J Histochem Cytochem* 15:713, 1967.
- Ofek I., Mirelman D., Sharon N. Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. *Nature* 265:623, 1977.
- Owen R. L., Jones A. L., Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles. *Gastroenterology* 66:189, 1974.
- Owen R. L., Nemanic P. Antigen processing structures of the mammalian intestinal tract: a SEM study of lymphoepithelial organs. *Scanning Electron Microscopy (1978) vol II* SEM Inc AMF O'Hare IL 60666, USA.
- Palay S. L., Karlin L. J. An electron microscopic study of the intestinal villus. 1. The fasting animal. *J Biophys Biochem Cytol* 5:363, 1959.
- Patt H. M., Quastler H. Radiation effects on cell renewal and related systems. *Physiol Rev* 43:357, 1963.
- Penn T. E., Patterson W. B., Eddy H. Protection of normal tissues during X-irradiation by temporary hypoxia. *Surg. Forum* 26:69, 1975.
- Pfeiffer C. J. Surface topology of the stomach in man and laboratory ferret. *J Ultrastructure Research* 33:252, 1970.
- Pfeiffer C. J. Scanning electron microscopy: A new tool for physiologic and pathophysiologic study of the digestive tract. *Biol Gastro-Enterol Tome IV*:273, 1971.
- Plattner H., Klima J., Mehnert A., Berger H. Quantitative and qualitative changes of structure and ultrastructure of intestinal epithelia during incubation in vitro. *Virchows Arch Abt B Zellpath* 6:337, 1970.
- Porter P. Discussion. A Ciba Foundation Symposium New York: North-Holland p. 65, 1972.
- Porvaznik M., Walker R. I., Gillmore J. D. Reduction of the indigenous filamentous microorganisms in rat ilea following - radiation. *Scanning Electron Microscopy (1979) III* SEM Inc AMF O'Hare IL 61666, USA.
- Puck T. T., Markus P. I. Action of X-rays on mammalian cells. *J Exp Med* 103:653, 1956.
- Quastler H. The nature of intestinal radiation death. *Radiat Res* 4:303, 1956.
- Quastler H., Sherman F. G. Cell population kinetics in the intestinal epithelium of the mouse. *Exp Cell Res* 17:420, 1959.
- Reeves R. J., Cavanaugh P. J., Sharpe K. W., Thorne W.

- A., Winkler W. A., Sanders A.P. Fat absorption studies and small bowel X-ray studies in patients undergoing Co⁶⁰ teletherapy and/or radium application. *Am J Roentgen* 94:848, 1965.
- Rijke R. P. C., van der meer-fieggen W., Galjaard H. Effect of villus length on cell proliferation and migration in small intestinal epithelium. *Cell Tissue Kinet* 7:577, 1974.
- Rijke R., Plaisier H., Hoogeveen A., Lamerton L., Galjaard H. The Effect of Continuous Irradiation on Cell Proliferation and Maturation in Small Intestinal Epithelium. *Cell Tissue Kinet* 8:441, 1974.
- Roswitt B., Malsky S. L., Reid C. B. Severe radiation injuries of the stomach, small intestine, colon and rectum. *Radiology* 114:460, 1972.
- Rubin P. H., Casarett G. W. *Clinical Radiation Pathology*, Vol. I. W. B. Saunders, 1968.
- Rydberg B., Johansson K. J. Radiation-induced DNA Strand Breaks and Their Rejoining in Crypt and Villous Cells of the Small Intestine of the Mouse. *Radiat Res* 64:281, 1975.
- Savage D. C., Dubos R., Schaedler R. W. The gastrointestinal epithelium and its autochthonous bacterial flora. *J Exp Med* 127:67, 1968.
- Savage D. C. Localization of certain indigenous microorganisms on the ileal villi of rats. *J Bact* 97:1505, 1969.
- Savage D. C., Mc Allister J. S., Davis C. P. Anaerobic bacteria on the mucosal epithelium of the murine large bowel. *Infection and immunity*, 4:492, 1971.
- Savage D. C. Survival on mucosal epithelia, epithelial penetration and growth in tissues of pathogenic bacteria. *Microbial Pathogen in Man and Animals. Symp Soc Gen Microbiol* 22nd; 25. 1972
- Smith D., Tyree E. Influence of X-irradiation upon body weight and food consumption of the rat. *Amer J Physiol* 177:25, 1954.
- Sténson S. Weight change and mortality of rats after abdominal proton and roentgen irradiation. A comparative investigation. *Acta Radiol Ther Phys Biol* 8:423, 1969.
- Stone H. B., Withers H. R. Metronidazole: Effect on radiosensitivity of tumor and normal tissue in mice. *J Nat Cancer Instit* 55(5): 1189, 1975.
- Strandquist M. Studien über die Kumulative Wirkung der Roentgenstrahlen bei Fraktionierung. *Acta Radiol Suppl.* 55:1, 1944.
- Strockbine M. F., Hancock J. E., Fletcher G. H. Complications in 831 patients with squamous cell carcinoma of the intact uterine cervix treated with 3000 rads or more whole pelvis irradiation. *Am J Roentgen* 108:293, 1970.
- Sullivan M. F. Gastrointestinal radiation injury. Report of a Symposium held at Richland, Wash., U.S.A. on September 25-28, 1966.
- Swift M.N., Taketa S. T., Bond V.P. Delayed gastric emptying in rats after whole and partial body X-irradiation. *Am J physiol* 182:479, 1955.
- Taketa S. T. Water-Electrolyte and Antibiotic Therapy against Acute (3- to 5-day) Intestinal Radiation Death in the Rat. *Radiation Research* 16:312, 1962.
- Takeuchi A., Zeller J. A. Scanning electron microscopic observations on the surface of the normal and spirochete-infested colonic mucosa of the Rhesus monkey. *J Ultrastructure Res* 40:313, 1972.
- Tannock G. W., Savage D. C. Influences of dietary and environmental stress on microbial populations in the murine gastrointestinal tract. *Inf Immun* 9:591, 1974.
- Tarpila S. Morphological and functional response of human small intestine to ionizing irradiation. *Scand J Gastroent* 6 suppl 12, 1971.
- Toner P. G., Carr K. E. The use of scanning electron microscopy in the study of the intestinal villi. *J Path* 97:611, 1968.
- Toner P. G., Carr K. E., Ferguson A., Mackay C. Scanning and transmission electron microscopic studies of human intestinal mucosa. *Gut* II: 471, 1970.
- Trier J. S. Studies on Small Intestinal Crypt Epithelium. II. Evidence for and mechanisms of secretory activity by undifferentiated crypt cells of the human small intestine. *Gastroenterology* Vol 47, 1964.
- Tsubouchi S., Matzuzawa T. Rapid radiation cell death and cell proliferation in the intestinal epithelium after 1000-rad irradiation. *Radiat Res* 57:451, 1974.
- Turesson I., Notter G. Skin reactions after different fractionation schedules giving the same cumulative radiation effect. *Acta Radiol Ther Phys Biol* 14:475, 1975.
- Upton A. C. *Radiation Injury*. The University of Chicago Press 1969.
- van der meer-fieggen W. Regulation of cell proliferation and differentiation in intestinal epithelium. Thesis Medische Faculteit te Rotterdam 1973.
- Waren S. L., Whipple G. H. Roentgen ray intoxication I. Bacterial invasion of the blood stream as influenced by X-ray destruction of the mucosal epithelium of the small intestine. *J Exp Med* 38:713, 1923.
- Vatistas S., Hornsey S. Radiation induced protein loss into the gastrointestinal tract. *Brit J Radiol* 39:547, 1966.
- Wiernik G. Radiation damage and repair in the human jejunal mucosa. *J Path Bact* 91:389, 1966.
- Wiernik G., Plant M. A. The origin and kinetics of Goblet cells in human jejunum during irradiation. *Brit J Radiol* 44:348, 1971.
- Withers H. R., Elkind M. M. Dose survival characteristics of epithelial cells of mouse intestinal mucosa. *Radiology* 91:998, 1968.
- Withers H. R., Elind M. M. Radiosensitivity and fractionation response of crypt cells of mouse jejunum. *Radiat Res* 38:598, 1969.
- Withers H. R., Elkind M. M. Microcolony survival assay for cells of mouse intestinal mucosa exposed to radiation. *Int J Radiat Biol* 17:261, 1970.
- Withers H. R. Regeneration of intestinal mucosa after irradiation. *Cancer* 28:75, 1971.
- Withers H. R., Chu A. M., Reid B.O., Hussey D. H. Response of mouse jejunum to multifraction radiation. *Int J Rad Onc Biol Phys* 1:41, 1975.
- Whittaker D. K., Drucker D. B. Scanning electron microscopy of intact colonies of microorganisms. *J Bacteriol* 104 (1):902, 1970.
- Zauder H.L. Response of radiated animals to anaesthetics and analgetics. Abstract References No 5683. In: The effects of radiation and radioisotopes on the life proces-

ses. US Atomic Energy Commission, Division of Technical Inform. Washington 1963.

Zetterqvist H. The ultrastructural organization of the columnar epithelial cells of the mouse jejunum. Thesis Stockholm Karolinska Institutet. 1956.

Peyer's patch.

Dome of a nodule surrounded by villi. 48

